The American Journal of Medicine

October 1952

The American Journal of Medicine

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The American Journal of Medicine

Vol. XIII OCTOBER, 1952 No. 4

Editorial

Clinical Studies

Treatment failures *in vivo* with antibiotics known to be effective *in vitro* usually are ascribed to inability to achieve effective concentrations of the antibiotic in critically infected areas or to the presence or development of resistant bacterial strains. This study emphasizes another contributory factor in connection with the use of penicillin: in older infections, organisms may no longer be multiplying and metabolizing very rapidly and so become insensitive to the mechanisms by which penicillin exerts its bacteriostatic or bactericidal effects.

This careful study deals with the recurrent problem of the relative significance of environment and heredity in the occurrence of rheumatic fever, a matter of continuing interest despite the advent of antibiotic prophylaxis and therapy. Poor housing and crowding were found again to be significantly greater in the rheumatic than in the control families. Heredity probably plays some role, presumably of inherited susceptibility or altered host response.

Rickettsialpox is not confined to New York City, four probable cases being reported from Philadelphia in this paper. It must be admitted that the diagnosis in some of these instances is not altogether secure but the important point is awareness of the disease and its characteristics.

Octamethyl pyrophosphoramide (OMPA) is one of the new and potent anticholinesterase agents being tried out in the treatment of myasthenia gravis. According to this report on the results in sixteen patients, OMPA permitted less limitation of activity than other drugs employed and was relatively free of significant side effects except on the gastrointestinal tract.

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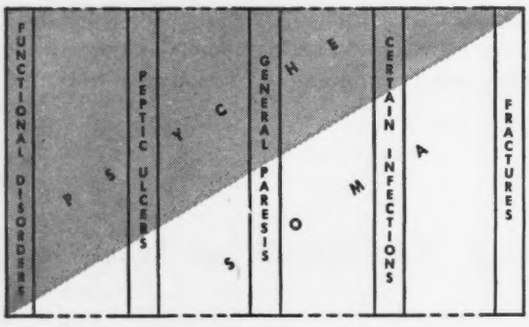
Therapy for Mixed Somatic and Psychic Complaints

Illness may be divided into:

a.) that which begins by a disturbance of function with physical breakdown (primary organic).

b.) that which begins in the psychic or emotional sphere and may eventually lead to physical breakdown (psychosomatic).

Stevenson[1] categorizes diseases according to the relative amounts of *psychic* and *physical* manifestations uncovered during interview with the patient. Burlingame's[4] classification, upon which the following chart is based, evolved from a similar thesis.



Diagnosis: people differ widely in their predisposition toward disturbances of emotional origin. The patient with greater constitutional predisposition develops illness under a relatively low level of stress; whereas the more stable individual can make good adaptation until exposed to a higher intensity of stress. For this reason examination should evaluate:

A.) Factors such as —
Emotional stability and family history.
Interpersonal relationships.
Life situations at work, in family and social areas. (Stevenson[1])

B.) *Complaints offered by patient* —

e.g. { lump in throat, substernal pain, palpitation, sweating, fatigue, sick to stomach, diarrhea, menstrual irregularities.

C.) *Complaints elicited by questioning patients*–

e.g. { admission of chronic anxiety, irritability, fear of illness and/or economic loss etc., difficulty in dealing with others.

D.) *Findings on examination of the patient:* muscular tenseness, moist skin, dry mouth, variable pulse rate etc. (Ebaugh[2])

Ebaugh[2] reports that somatic manifestations of such illness are intimately related to the balance between sympathetic and parasympathetic nerve tonus. Since emotions relayed via the hypothalamus, activate both adrenergic and cholinergic discharges, continuous emotional stress and anxiety result in functional disturbances.

Treatment of such conditions, therefore must be based on two methods of management:

1.) *psychotherapeutic* — to help patient adjust to stressful situations with minimal emotional trauma.

2.) *medicinal* — to relieve the patient's symptomatic distress and concern over his illness, thereby making him more amenable to psychotherapy.

Bellergal — is particularly suited as an adjunct to psychotherapy for functional disorders. MacFadyen[3] explains the rationale of this therapy as follows. Bellergal contains:

1.) *Ergotamine tartrate and Bellafoline*—these block sympathetic and parasympathetic impulses respectively, thus inhibiting transmission of stimuli which originate from emotional centers.

2.) *phenobarbital* — which inhibits the exaggerated response of the neurotic patient to stressful situations.

This combination ". . . subdues the central, sympathetic and parasympathetic activities in such a manner that the dominance of any one division is gradually decreased to a point where normal balance is re-established."[3]

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4. Burlingame, C.: Connecticut M. J. *14:* 493, 1950.

The Sandoz Scientific Department has prepared a booklet entitled *Atlas of Emotional Disorders,* useful for explaining the basic origin of functional disorders of the various systems. These are available by writing to:

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Research Society Abstracts

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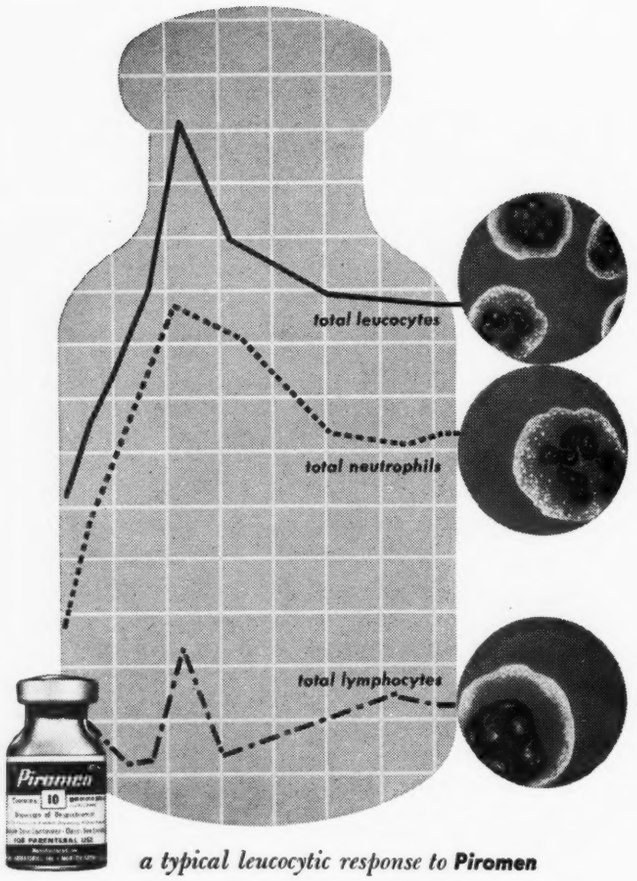

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1. Sturgis, C. C.: Postgraduate Med. 5:300, 1949.

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1. Douglas, H. S.: West. J. Surg. 59:238, 1951.

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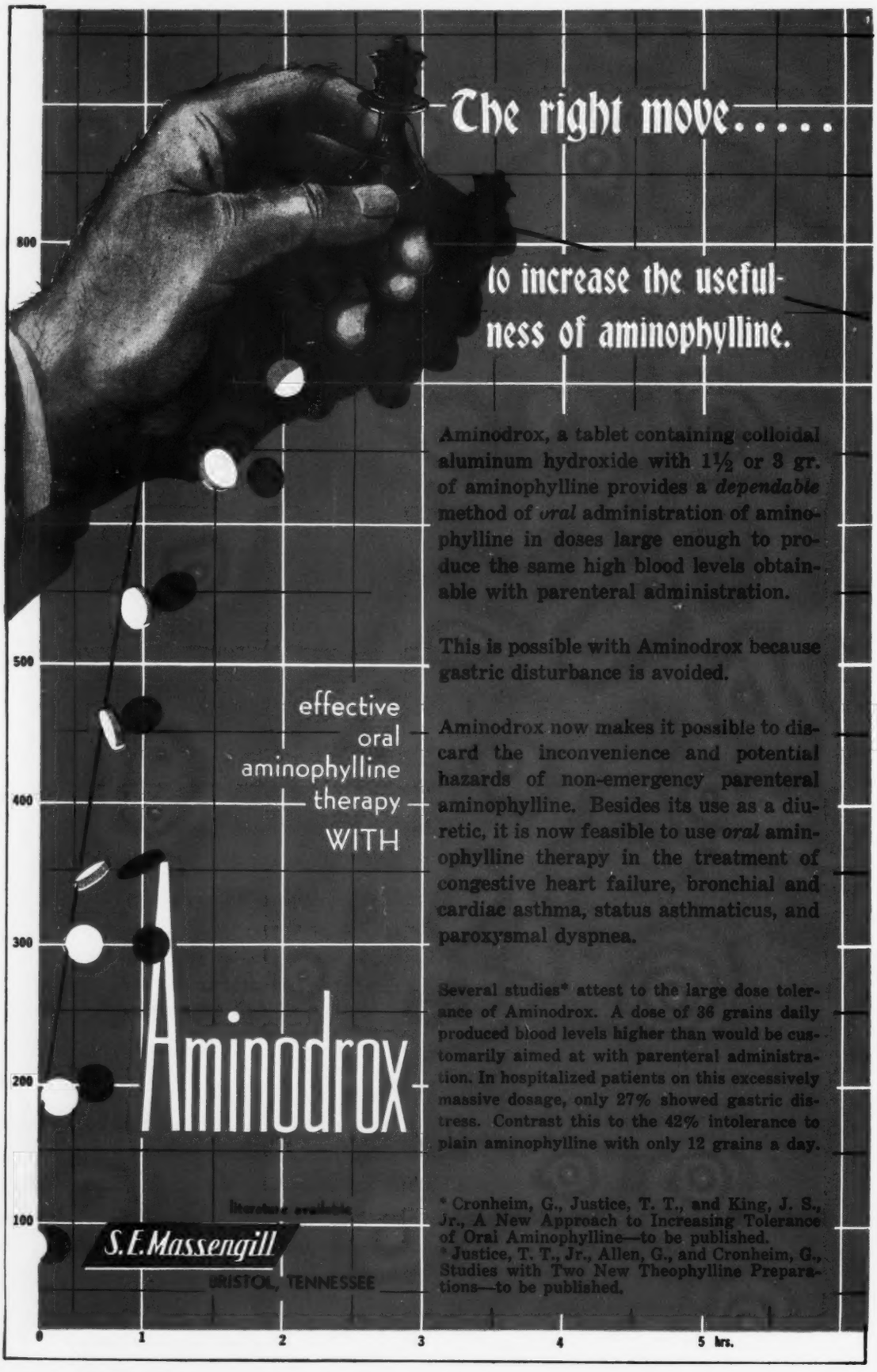
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* Cronheim, G., Justice, T. T., and King, J. S., Jr., A New Approach to Increasing Tolerance of Oral Aminophylline—to be published.
* Justice, T. T., Jr., Allen, G., and Cronheim, G., Studies with Two New Theophylline Preparations—to be published.
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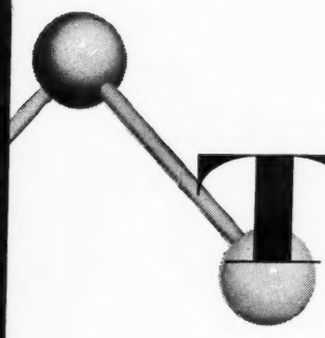

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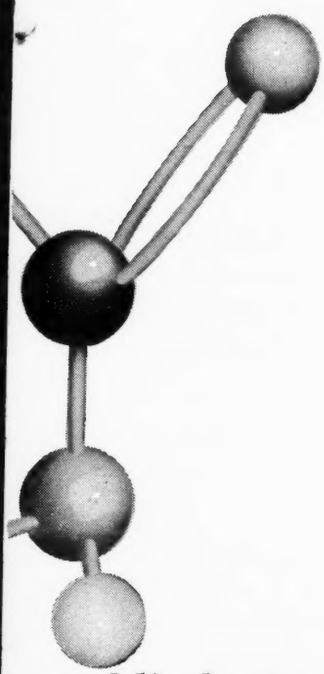

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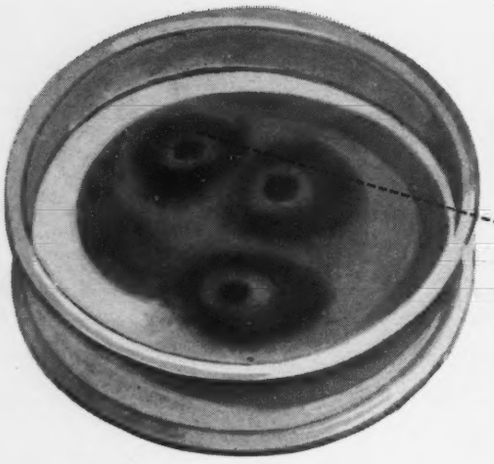

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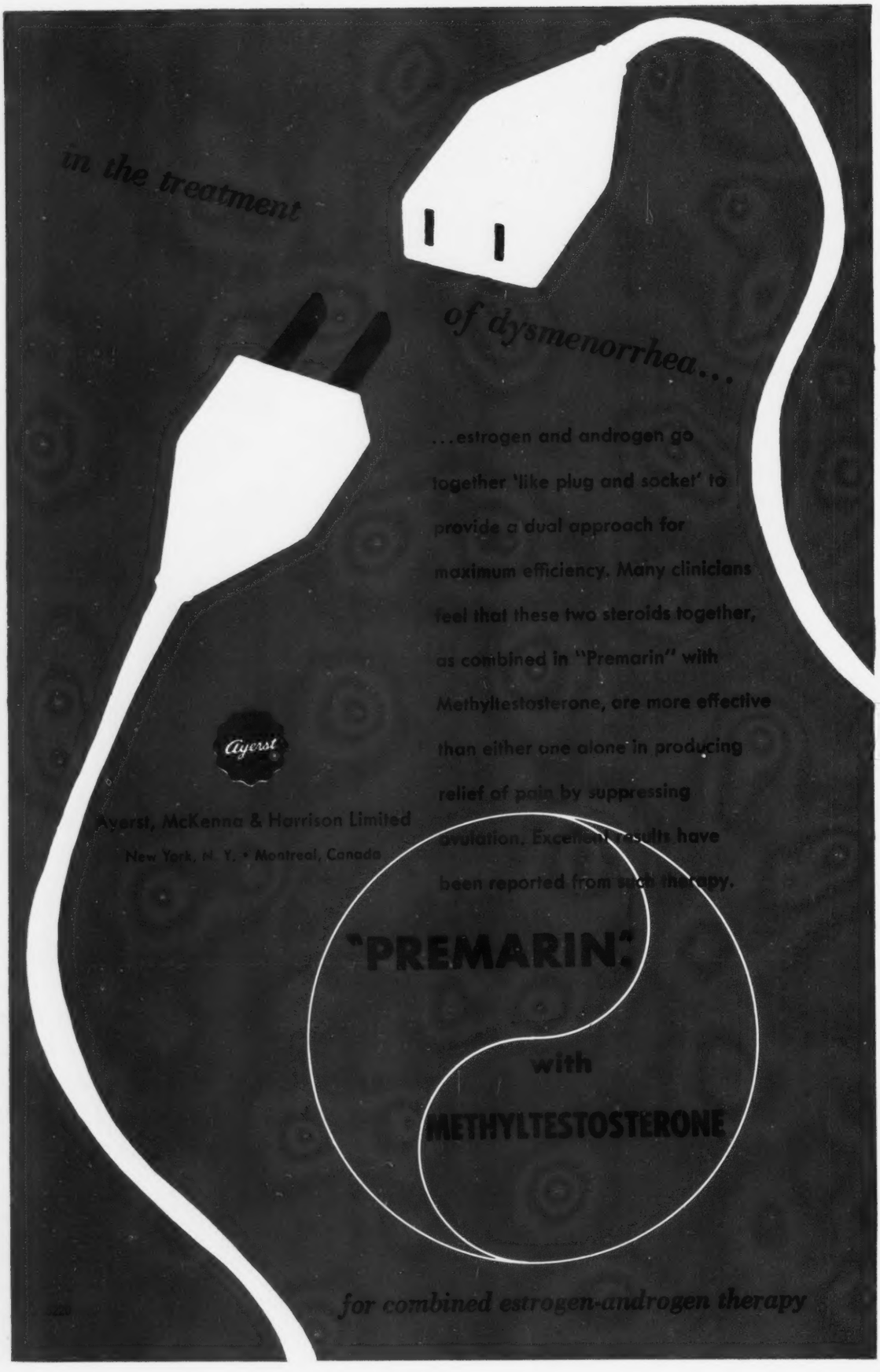
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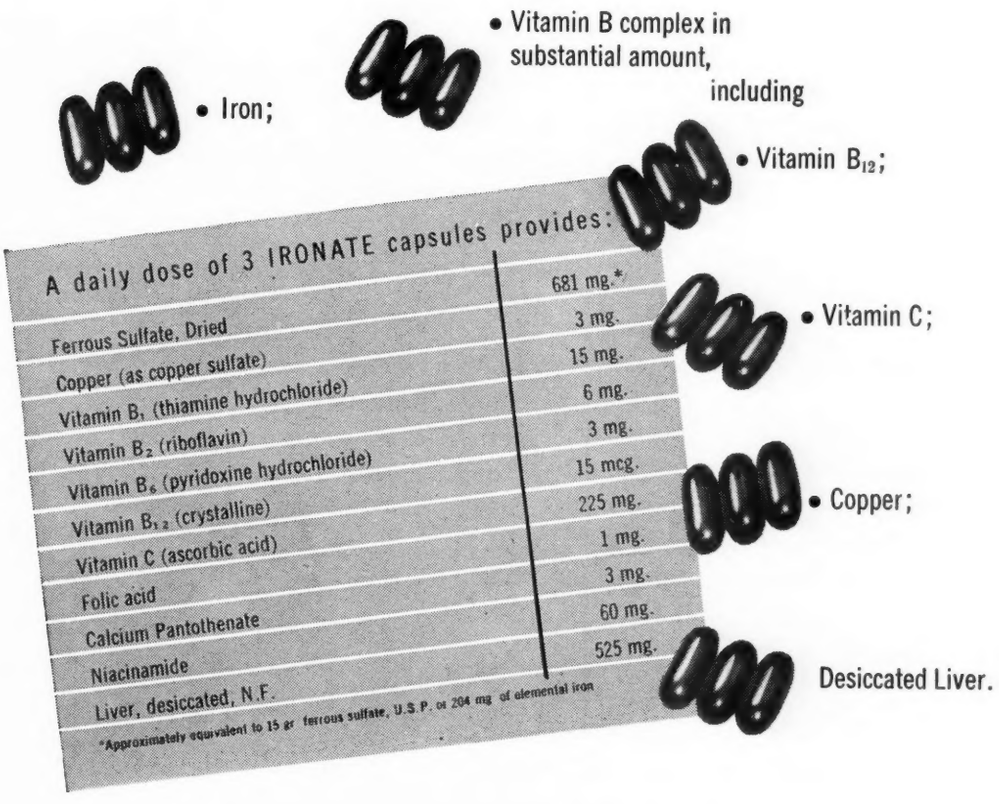

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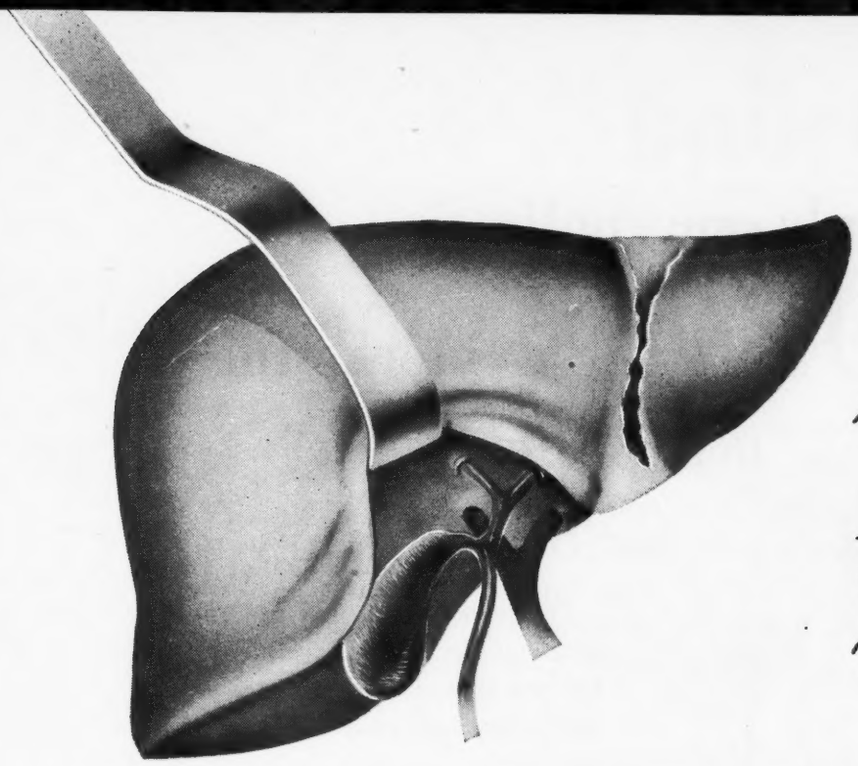

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Boller, R.; Rottino, A., and Pratt, G. H.: Therapeutic Action of Muscle Adenylic Acid on Ulcers and Dermatitis Associated with Varicose or Phlebitic Veins; Follow-up Report, Angiology 3:260, 1952.

The following papers referring to adenylic acid, muscle adenylic acid, and adenosine-5-monophosphate are all based on Bischoff's MY-B-DEN and its use in varicose vein complications:

Lawrence, E. D.; Doktor, D., and Sall, J.: Muscle Adenylic Acid: A Clinical Study of Its Effect, Angiology 2:405, 1951.
Rottino, A.: Boller, R., and Pratt, G. H.: Therapeutic Action of Muscle Adenylic Acid on Ulcers and Dermatitis Associated with Varicose or Phlebitic Veins; Preliminary Report, Angiology 1:194, 1950.

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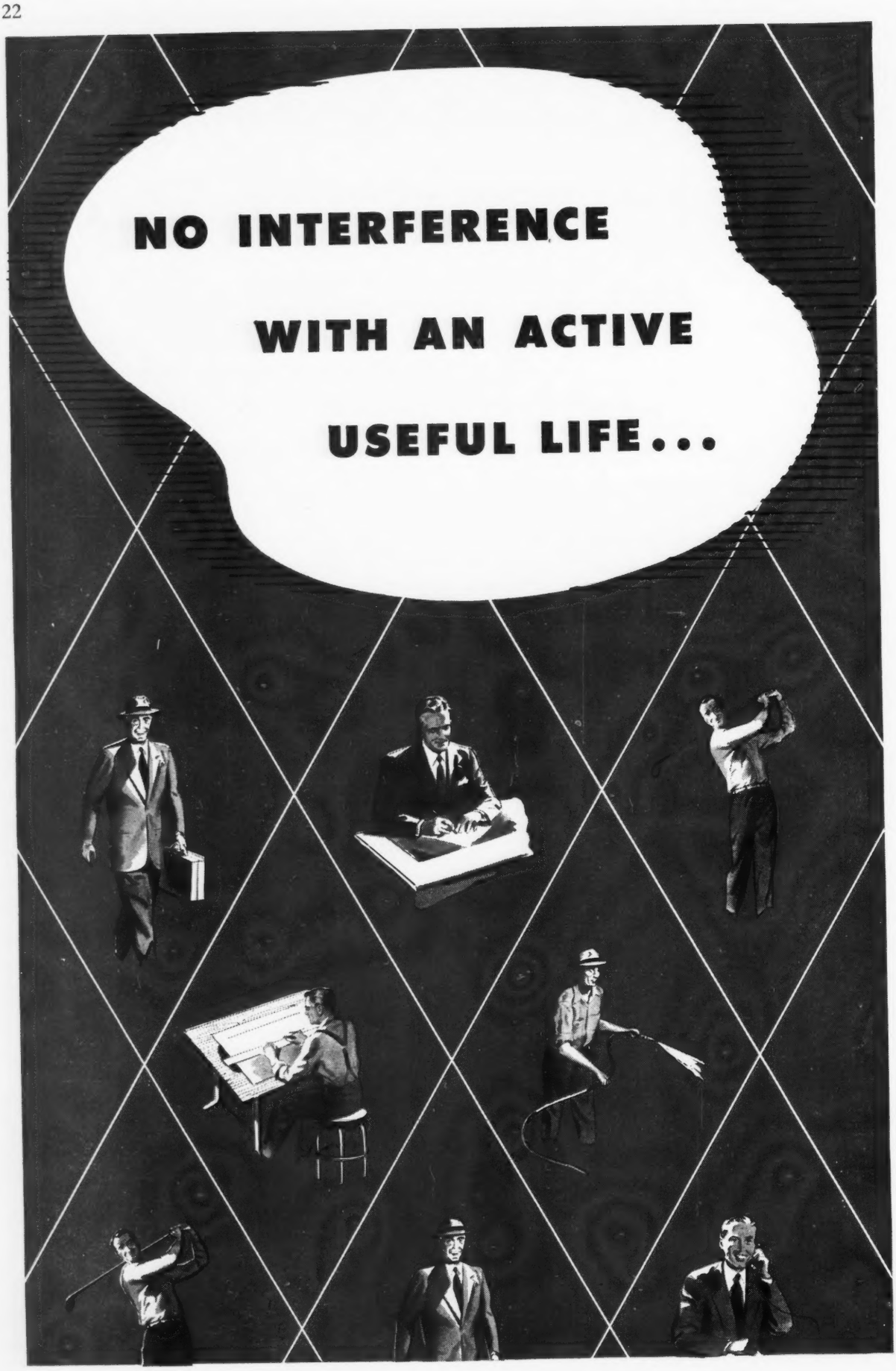
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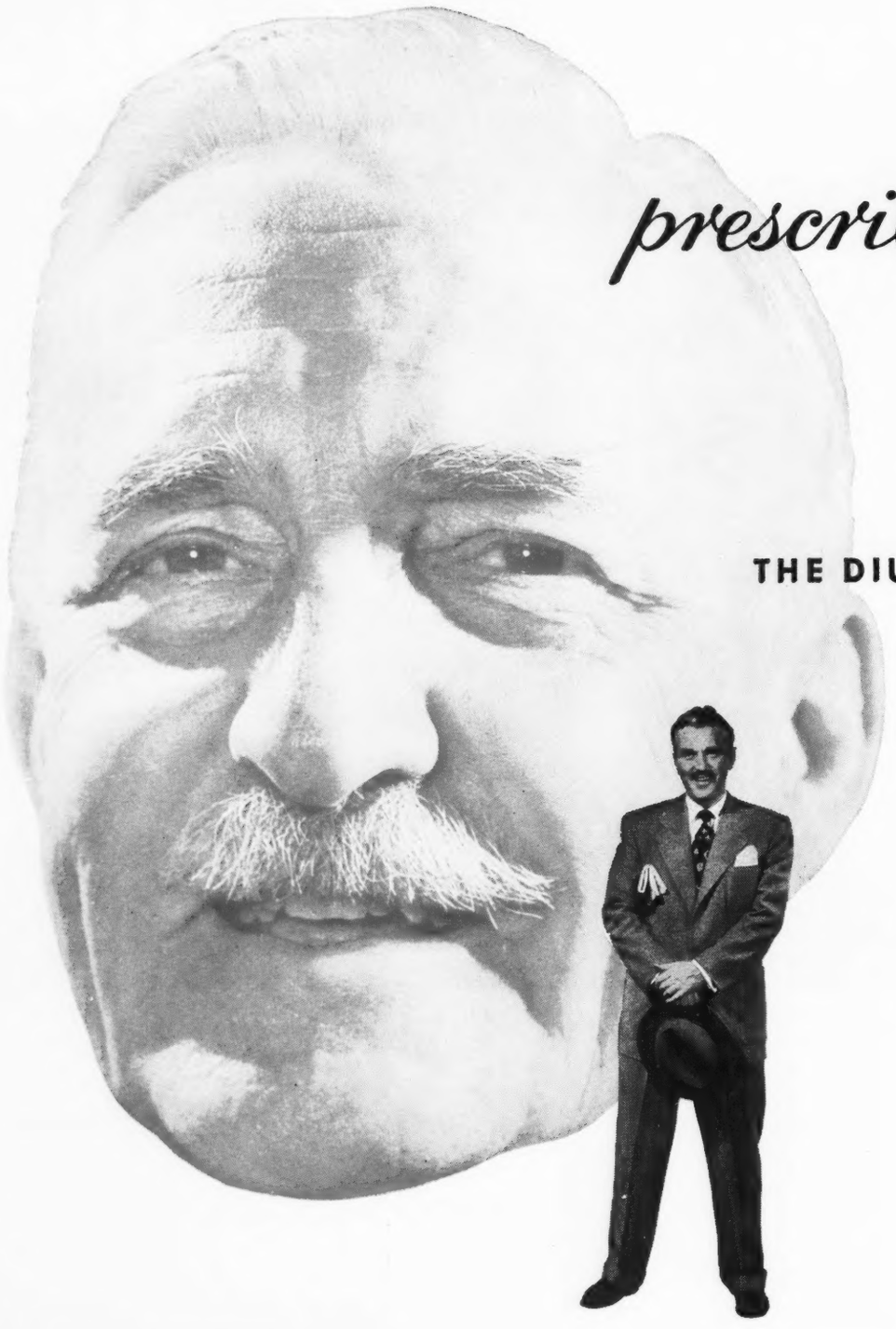

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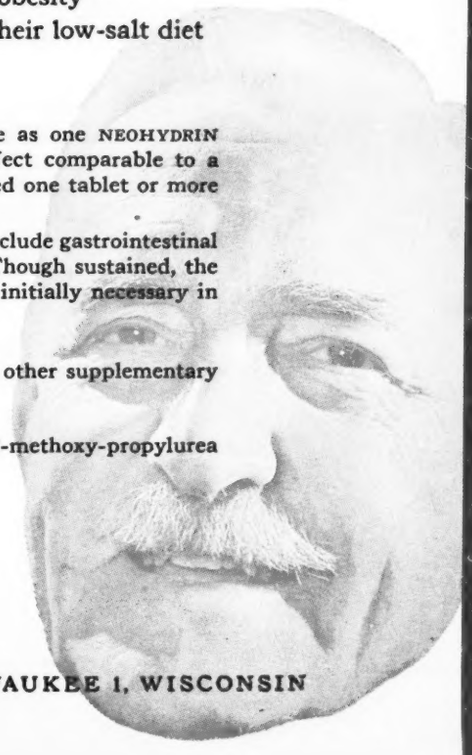

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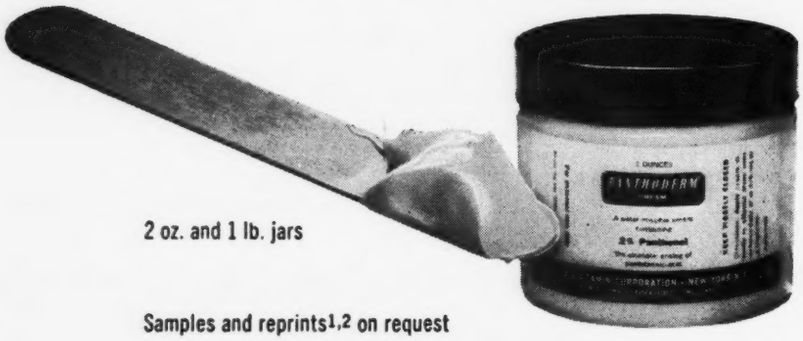

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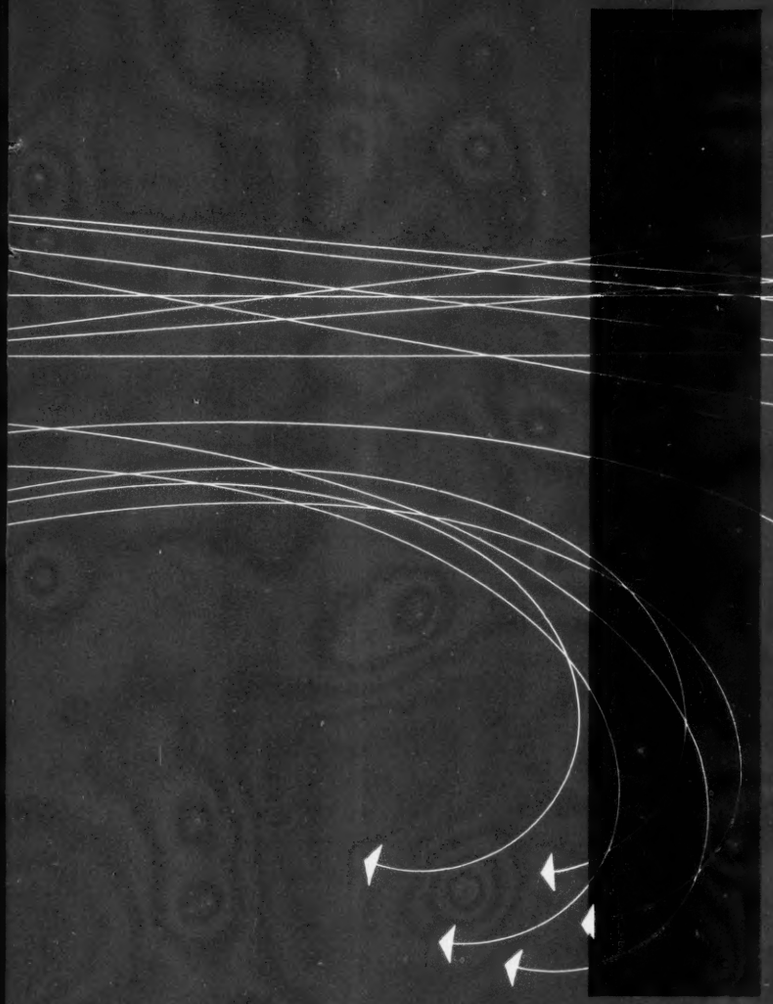

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The American Journal of Medicine

VOL. XIII OCTOBER, 1952 NO. 4

Fainting

SUDDEN temporary loss of consciousness has always been of interest to the physician. Syncope may be a symptom of little significance or it may be of extreme importance. Soma Weiss wrote a classic review of this subject in Oxford Medicine in 1939.[1] It is worth while to review the additions and changes in emphasis that he would make to this article if he were rewriting it in 1952.

Much has been added to our knowledge of the circulatory changes in the common faint. In the horizontal position the striking fall in arterial pressure is accompanied by a marked redistribution of blood flow. The skin flow falls to nearly zero and the muscle flow rises.[2] The cardiac output may show little change, indicating that the fall in blood pressure is the result of over-all arteriolar dilatation.[2,3] Heart action is, however, actually inhibited because ordinarily a fall in arterial pressure serves as a stimulus to increase the output. It is assumed that this inhibition is neurogenic in origin. Right atrial pressure may not change during the fall in arterial pressure.[3]

More emphasis is now placed on the role of hyperventilation in setting the stage for vasodepressor syncope (common faint).[4] In the recumbent position overbreathing will lead to changes in mental status but does not usually cause unconsciousness. In the upright position it acts frequently as the trigger mechanism to set off the vasodepressor reaction characteristic of the common faint. Hyperventilation may be of importance in the fainting that occurs on exertion with any severe form of congestive heart failure, or with severe aortic stenosis without overt failure.

The common faint is the result of a widespread stimulation of both the sympathetic and parasympathetic systems. The afferent stimuli may be from the emotional content of thought or from any sensory nerve. The sweating, pallor, nausea, dilated pupils, increased intestinal activity, slow pulse and fall in arterial pressure are all symptoms of this widespread stimulation of the autonomic nervous system. The fall in arterial pressure does not produce these symptoms as they may all be present before the arterial pressure falls. Actual loss of consciousness while the patient is standing does seem to result from cerebral ischemia secondary to the hypotension. The decrease in urine output is caused by neurogenic discharge of antidiuretic hormone by the postpituitary gland.[5] It is possible that the antidiuretic hormone concentration may be increased sufficiently to account for some of the intense skin vasoconstriction, but no observations are available on this point.

Rarely, a pregnant woman faints whenever she lies flat on her back.[6,7] No symptoms appear when lying on her side. This reaction probably represents a summation of two events: (1) stimulation of sensory pathways in the abdomen by weight of the uterus, and (2) pooling of blood in the lower extremities because of the uterus pressing on the inferior cava.

Neurogenic postural hypotension of a major degree has become more common. Diabetic neuropathy is now the most common cause. In the diabetic patient the disturbance is of two types: (1) lack of arteriolar constriction when the patient stands in the upright position lead-

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ing to a low peripheral resistance and a relatively normal cardiac output; (2) lack of venous constriction leading to a low cardiac output with normal peripheral resistance.[8] Neurogenic postural hypotension, even when severe enough to cause loss of consciousness, is not usually accompanied by the signs of generalized overactivity of the autonomic nervous system characteristic of the common faint. This is further evidence that the symptoms of the faint, except for the loss of consciousness, are not the result of the hypotension produced by the arteriolar dilatation.

Fainting in tetralogy of Fallot is now better understood. Exercise increases the removal of oxygen in the muscles and causes markedly unsaturated venous blood to enter the right heart. A portion of this black blood passes through the shunt to the left ventricle and out to the body. As the blood flow through the lungs is little increased, the arterial oxygen saturation drops rapidly and the patient loses consciousness from cerebral anoxia. The anoxia is produced by the marked arterial oxygen unsaturation and not by a reduction in cerebral blood flow.

The syndrome of pulmonary hypertension due to pulmonary arterial disease is frequently accompanied by fainting.[9] These subjects on exercise are unable to increase their cardiac output, become fatigued, gray and lose consciousness. Syncope may or may not be associated with changes in cardiac rhythm. It is interesting to speculate on whether, in addition to a reduced cerebral blood flow, respiratory alkalosis is important in these patients. Many of them have good function of the lungs as a bellows and little disease of the alveolar and capillary walls. The marked overbreathing produced by light exercise should cause a marked lowering of CO_2 content of arterial blood and a rise in pH. Actual measurements are needed to clarify the magnitude of such changes.

The incidence of tussive fainting has increased as we have asked more patients whether they ever lose consciousness with coughing. Tussive fainting is produced by the same mechanism that children use to produce unconsciousness. When one compresses the thorax of a subject who has taken a deep breath and has closed his glottis, circulation is blocked because of the high pressure produced in the thorax and syncope occurs. If one has a disease which prevents rapid emptying of the thorax, a paroxysm of coughing may build up high enough intrathoracic pressure to prevent normal circulation within the thorax and syncope may occur.[10] This type of faint is frequently seen in severe emphysema and obstructive lesions of the trachea, such as aneurysms and tumors. It is occasionally seen in patients with hysteria who close the glottis and bear down hard.

We used to think that the sudden loss of consciousness in patients with paroxysmal tachycardias was commonly the result of a low cardiac output secondary to a reduced diastolic filling time. Better history-taking has shown that in some instances the loss of consciousness occurs either at the beginning or end of the attack.[11] Cardiac standstill just before the abnormal rhythm begins or as it stops is probably the more common cause of the syncope.

With the advent of modern cardiac surgery, more attention is paid to the possibility of pedunculated tumors or ball valve thrombosis as causes of sudden loss of consciousness. If mitral stenosis is present, sudden loss of consciousness always raises the question of a ball valve thrombus. Signs of pulmonary hypertension and left auricular enlargement without the rest of the picture of mitral stenosis raises the question of a tumor of the auricle. If it is pedunculated, syncope may occur.

In the last few years the importance of CO_2 retention as a cause of confusion and loss of consciousness has been better appreciated. Patients in respirators and those with advanced pulmonary disease may have confusion and stupor as the basis of CO_2 retention. Of particular interest is the unfavorable effect of oxygen on some of these patients with CO_2 retention and marked arterial oxygen unsaturation. In these patients the reduced oxygen tension is serving in a large part as the stimulus to maximal ventilatory effort and, in spite of this, CO_2 tension is already greatly elevated. On breathing high concentrations of oxygen in the inspired air, the arterial oxygen tension rises, the ventilating volume decreases as the anoxic drive ceases and acute CO_2 poisoning occurs with stupor, coma and eventually death.[12,13]

EUGENE A. STEAD, JR., M.D.

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Clinical Studies

Experimental Approach to the Problem of Treatment Failure with Penicillin*

I. Group A Streptococcal Infection in Mice

HARRY EAGLE, M.D.
Bethesda, Maryland

EVEN large dosages of penicillin continued for long periods of time may sometimes fail to effect cure in infections caused by organisms which, judged by their *in vitro* sensitivity, should have been readily controlled. Some of these failures may reasonably be attributed to the fact that the drug does not attain the effective concentration in a walled-off, suppurating or necrotic focus, or in a cavity into which the drug can diffuse only slowly and to a limited degree. In others, the causative organism can be shown to have become resistant to the drug during its prolonged administration. In many cases, however, the treatment failure cannot be so simply explained. Thus in subacute bacterial endocarditis, although 100 per cent "cure" has been observed in several small series of cases,[1,2] usually from 10 to 40 per cent of the patients are not cured even when large doses of penicillin are given over a period of many weeks.[3–8] The treatment failures observed in early syphilitic infection are even more surprising. It is true that Treponema pallidum is killed rather slowly by penicillin.[9–11] It is, however, one of the most sensitive organisms yet studied in terms of the minimal effective concentrations. As little as 0.002–0.005 μg. of penicillin per cc. has been estimated to exert a definite if slow treponemicidal effect both *in vitro*[9] and *in vivo*.[10,11] Nevertheless, from 5 to 15 per cent of the cases of primary or secondary syphilis are not cured on any schedule of treatment yet tried, using either aqueous penicillin or procaine penicillin in oil with aluminum monostearate, and including schedules in which treatment is extended over a period of one to eight weeks, with a total of up to 9.6 million units.[12–16] In leptospiral infections although the organism is sensitive *in vitro* to concentrations of 0.1 units penicillin per cc.,[17] the therapeutic results, particularly in the later stages of the disease, have been disappointing.[18]

The present paper represents an attempt to study in experimental streptococcal infections of mice some of the factors which may be responsible for these paradoxical treatment failures. It had previously been shown[19,20] that in the experimental animal, the larger the inoculum of pneumococci, streptococci or Treponema pallidum, the larger were the single dosages of penicillin required to effect cure, i.e., the longer the time for which the drug had to be provided at effective levels. Similarly, the longer the time which was allowed to elapse between inoculation and treatment, the larger was the curative dose of penicillin. In large part, the latter phenomenon reflects the multiplication of the organisms in the interval between inoculation and treatment. However, in mice inoculated with group A or group B streptococci, if twenty-four hours were allowed to elapse between inoculation and treatment, no single dose of aqueous penicillin even up to the toxic range then sufficed to effect cure.[19] As will be shown, the latter treatment failures are not due merely to the large number of organisms present in the infected host. Additional factors supervene in the older infection to reduce the bactericidal action of the drug to a striking degree.

METHODS AND MATERIALS

The strain of organism used in these studies was the group A C-203 strain.† Its virulence was

† This was typed by Dr. Rebecca Lancefield and found to have the M antigen of type 3 and the T antigen of type 1. Her assistance is gratefully acknowledged.

* From Section on Experimental Therapeutics, National Microbiological Institute, National Institutes of Health (Public Health Service, Federal Security Agency), Bethesda, Md.

kept at a high level throughout the duration of these studies by passage through mice repeated two to three times weekly. The organisms were grown on beef heart infusion broth enriched with 2 per cent horse blood. No more than one test tube transfer intervened between the isolation from the mice and the use of the culture in the experiment, and cultures were less than four hours old. The number of organisms inoculated was controlled by darkfield microscopic count (with a simultaneous determination of the number of organisms per chain), and was checked by plating out in blood agar. When indicated, chains were broken up by grinding the culture or culture-dilution in a Waring blender for thirty seconds. Mice weighing 17.5 to 22.5 gm. were inoculated into the thigh muscle of the hind leg. Treatment, whether with aqueous sodium penicillin or procaine penicillin in oil* gelled with 2 per cent aluminum monostearate, was also given intramuscularly in the opposite leg or in the front shoulder muscle. Volumes of inoculum and of drug were 0.1 to 0.2 cc. The dosages of the procaine salt have been expressed as mg./kg. of the sodium salt, assuming 1,667 units = 1 mg. To estimate the number of organisms surviving in the muscle the skin was dissected away, the muscle and bone were removed aseptically, placed in 50 cc. of 2 per cent blood broth and emulsified for one and one-half minutes in a Waring blendor. Three cc. of this emulsion were used in plating, and when large numbers of organisms were anticipated, serial 40-fold dilutions were similarly plated. In determining mortality the experimental animals were kept for observation† for twenty-one days.

EXPERIMENTAL

Effect of the Age of the Infection on the Rate of Bactericidal Action of Penicillin in Vivo. When mice were inoculated intramuscularly with a small number of group A streptococci, the number of organisms in the muscle at first rapidly increased. In one experiment (open circles in Fig. 1) a median number of 590 colonies were recovered from the muscle immediately after inoculation with 1,000 organisms. In three hours this had increased to a median level of 10,000 organisms, after six hours to 225,000 and after nine hours to 5,000,000. Only a moderate increase was noted in the following fifteen hours, with a median number of 6 and 40 million organisms in the muscle at the twelfth and twenty-fourth hour, respectively.

At each of these time periods groups of mice were treated with a single dose of procaine penicillin in oil with aluminum monostearate, 0.1 cc. of a suspension containing 5,000 units per cc. Subgroups were then sacrificed one, two, four and eight hours after treatment, and the inoculated muscle emulsified and subcultured to determine the number of residual viable organisms. The effects of treatment are shown by the solid circles of Figure 1.

In the first six hours of the infection (sections A and B), during which the number of organisms in the muscle was rapidly increasing but had not yet exceeded 10^6, penicillin exerted a rapid and striking bactericidal effect. Within one hour after treatment the number of organisms in the muscle had decreased by a factor of 2 to 3 logs, and within two hours by a factor of approximately 4 logs, i.e., down to 1/10,000th of the original number. Corresponding to this rapid bactericidal effect there were no deaths in groups of ten mice treated three or six hours after inoculation.

A quite different result was observed when treatment was delayed until the twelfth hour of the infection (section C in Figure 1), when the number of organisms in the muscle had increased to a median of 6,000,000. The same dose of penicillin which had previously effected rapid sterilization now exerted only a slow and irregular bactericidal effect. Even eight hours after treatment the muscles still harbored from 300 to 5,000,000 viable organisms. A qualitatively similar effect was observed when mice were treated twenty-four hours after the infection (section D). Only in approximately one-fourth of the latter animals did penicillin exert a definite bactericidal effect, and in the others the number of organisms did not change significantly after treatment. The mortality in animals treated twelve and twenty-four hours after inoculation was 9/12 and 5/10, respectively, not significantly different from that observed in untreated controls (26/30).

The markedly retarded bactericidal action of penicillin in the older infections was shown again in the experiment of Figure 2 which, in the same

* I am indebted to the Bristol Laboratories, Syracuse, N. Y., for the large supply of the gelled peanut oil used in these experiments.

† The assistance of Mr. Ralph Fleischman and Miss Mina Levy in these experiments is gratefully acknowledged.

animal, contrasts the effect of the aqueous sodium salt in freshly inoculated muscles and in muscles inoculated twenty-four hours previously.

It was apparent from these and many similar experiments that in the freshly inoculated animal penicillin exerted a rapid bactericidal action, effecting sterilization and cure in a period of one and one-half to six hours (depending on the number of organisms inoculated), while in animals treated twelve and twenty-four hours after inoculation a single injection of procaine penicillin in oil with monostearate or 4 injections of the sodium salt no longer sufficed. Its bactericidal action was now so slow that daily treatment with the procaine suspension had to be continued for a period of six to eight days in order to effect sterilization and cure. (cf. Table 1.)

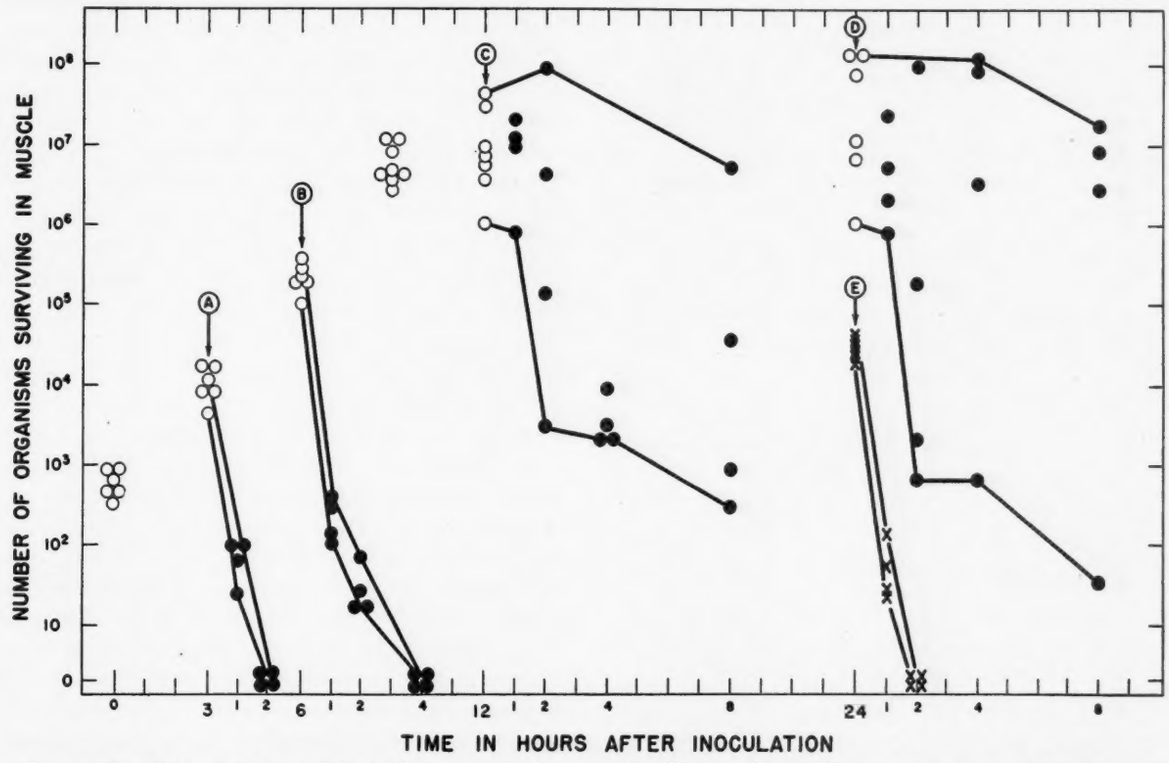

FIG. 1. The effect of the age of the infection on the therapeutic efficacy of penicillin in a streptococcal infection of mice. Mice were inoculated into a hind leg muscle with 1,000 group A streptococci (microscopic count) contained in 0.1 cc. Three, six, twelve or twenty-four hours later separate groups of animals were treated intramuscularly (indicated by ↓ in figure) with 0.1 cc. of procaine penicillin in oil with 2 per cent aluminum monostearate, at 5,000 units/cc. (25,000 units/kg.). The open circles in the figure show the number of organisms in the muscle at the time of treatment; the solid circles show the effect of treatment. Immediately before the twenty-four hour treatment a number of animals were re-inoculated into the opposite leg with 20,000 organisms. The effect of treatment on the number of organisms surviving in that leg muscle [(E)—(x-x)] is to be contrasted with the effect in the leg inoculated twenty-four hours previously [(D)—(o-o)].

Time between inoculation and treatment: (A), 3 hours; (B), 6 hours; (C), 12 hours; (D), 24 hours;
Mortality after twenty-one days: (A), 0/10; (B), 0/10; (C), 9/12; (D), 5/10; controls, untreated, 26/30.

Possible Explanations for the Retarded Bactericidal Action of Penicillin in Older Infections. A number of possible explanations were considered for the altered therapeutic response observed in older infections:

1. *Impaired host participation:* Conceivably, the therapeutic action of penicillin may involve the participation of as yet unidentified host factors, and that host participation might be curtailed in the sick animal with an older infection. This possibility seemed unlikely in view of the evidence[21,22] that in most experimental infections the therapeutic action of penicillin rests primarily on its direct bactericidal action, and continues for just as long as the drug remains at effective concentrations at the focus of infection.

In the present experiments the possibility that a systemic host factor had been impaired in the older infection was excluded by the fact that

when mice which had been infected twenty-four hours previously were re-inoculated in the opposite leg immediately prior to treatment, the bactericidal action of the drug was promptly manifest in the freshly inoculated muscle, but was not apparent in the muscle inoculated twenty-four hours previously (contrast sections D and E in Figure 1, and contrast the open and closed circles in Figure 2).

TABLE 1

TIME REQUIRED TO EFFECT CURE WITH PENICILLIN IN ESTABLISHED STREPTOCOCCAL INFECTIONS IN MICE *

No. of Daily Treatments with Penicillin	No. of Viable Organisms Surviving in the Muscle †	Mortality
0	125,000,000 6,700,000 107,000,000 4,500,000 93,000,000 4,100,000 8,600,000 4,000,000	20/20 = 100%
1	51,000,000 5,400,000 21,000,000 5,300,000 6,800,000 400,000	10/10 = 100%
2		19/20 = 95%
4	1200, 250, 50, 33, 17, 0	9/20 = 45%
6		3/20 = 15%
8	0, 0, 0, 0, 0, 0	0/19 = 0%
12		0/17 = 0%

* Mice were inoculated intramuscularly with 20,000 organisms. Twenty-four hours later, the animals were treated in the opposite leg with 0.15 cc. of procaine penicillin at 10,000 units per cc.; and this treatment was continued daily for the periods of time indicated in the table.

† The mice subcultured after only one treatment with penicillin were tested three days after the injection of the drug. The other groups were subcultured twenty-four hours after the last treatment indicated.

2. *Number of organisms:* The possibility that the striking decrease in the rate of bactericidal action of penicillin in the older infection might rest primarily on the large numbers of organisms then present was controlled by inoculating mice with varying numbers of organisms and then treating immediately. As shown in Figure 3, when the inoculum was so varied from 10,000 to 50,000,000 organisms, there was no demonstrable effect on the rate of bactericidal action of penicillin. On the other hand, when animals were allowed to go for twenty-four hours before treatment, there was the usual striking reduction in the rate of bactericidal action of the drug, this despite the fact that the muscle at the time of treatment contained fewer viable organisms than some of the freshly inoculated muscles successfully treated the day before. In another type of experiment (cf. Fig. 5) when a large number of organisms was inoculated, the bactericidal action was rapid when the mice were treated immediately or one and one-half hours after inoculation. However, if treatment was delayed for three hours, the therapeutic effect of penicillin was strikingly reduced, this despite the fact that there had been only an insignificant degree of multiplication in the intervening one and one-half hours. It seemed clear that the number of viable organisms *per se* was not the factor responsible for the relative inefficacy of penicillin in the older infections.

3. *Diffusion of penicillin into the muscle:* The possibility was considered that in the older infections, when there had been considerable inflammation, the diffusion of penicillin into the muscle might have been reduced to the degree that the concentration at the focus of infection was less than that necessary to kill the organism at the maximal rate. A priori, this seemed improbable because of the extreme sensitivity of this particular strain of streptococcus to penicillin.[23] A concentration of as little as 0.004 μg./cc. exerts a definite bactericidal action *in vitro*, 0.008 μg./cc. sterilizes suspensions containing 1 to 10 million organisms/cc., and 0.064 μg./cc. kill the organisms at the maximum rate. To study the factor of diffusion directly animals were inoculated with 10^3 organisms and treated twenty-four hours later, either with a single intramuscular injection of procaine penicillin at 45 mg./kg. (0.15 cc. of a suspension containing 10,000 units/cc.) or with aqueous sodium penicillin at 200 mg./kg., repeated every two hours for the duration of the experiment. At the latter dosage of aqueous sodium penicillin the serum concentration rises to a median peak of 200 μg. within fifteen minutes, and falls to a median level of only 2.5 μg./cc. after two hours.[24] For the duration of the entire experiment the serum concentration therefore remained in excess of 1 μg./cc. in most of the animals. In order to determine the concentration of penicillin in the muscle animals were exsanguinated by cardiac puncture fifteen minutes after the injection of

the drug, the inoculated muscle was removed, weighed, emulsified and its penicillin content assayed by a method previously described.[25] The muscle was found to contain 56 to 68 μg./gm., 10,000 times the minimal effective concentration, and some 50 to 100 times greater than the concentrations afforded by the injection of procaine penicillin. Nevertheless, as shown by the solid points in Figure 5, these massive doses of sodium penicillin were no more effective in the treatment of a twenty-four-hour infection than a single injection of the procaine salt in oil. It is true that the over-all penicillin concentration in the muscle does not necessarily reflect the concentration at a microscopic inflammatory focus in which the capillaries may already have thrombosed. However, one may reasonably assume that if, immediately adjacent to such a focus, penicillin were present in a concentration 10,000 times the effective level, enough would eventually have diffused into the area to effect a bactericidal action were the presence of drug the only limiting factor. In freshly inoculated mice, however, (0–0 and Δ–Δ in Figure 4), both preparations effected an equally rapid bactericidal action, sterilization and cure.

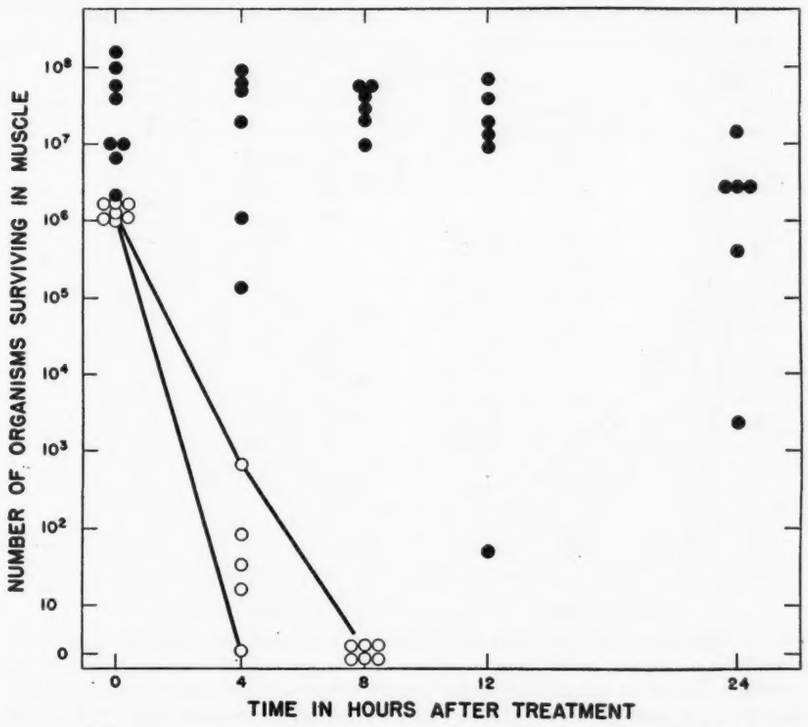

FIG. 2. The effect of the age of the infection on the therapeutic efficacy of penicillin, contrasting, in the same animal, the bactericidal action of penicillin in a freshly inoculated muscle (o-o) and in a muscle inoculated twenty-four hours previously (●-●). Mice were inoculated into the left thigh muscle with 10^3 streptococci. Twenty-four hours later the opposite leg was inoculated with 2×10^6 organisms, and the animals were immediately treated into a shoulder muscle with 0.15 cc. of aqueous sodium penicillin at 25 mg./cc. (188 mg./kg.). Treatment was repeated four, eight and twelve hours later. The contrasting therapeutic response in the two muscles is evident in the figure.

4. *The metabolic activity of the organisms as a factor conditioning the therapeutic action of penicillin to an important degree:* It is well established that penicillin kills bacteria most effectively *in vitro* only if the bacteria are in an environment which permits their multiplication.[26–31] The factor which determines the susceptibility of dividing bacteria to penicillin is probably not the actual continuing multiplication of the organisms, but rather the fact that they are actively metabolizing a favorable medium. As was clearly shown by Chain and Duthie,[30] if previously resting bacteria are placed in an environment conducive to growth and multiplication, the bacterial action of penicillin becomes evident

quickly before there had been a significant degree of multiplication.

It is a reasonable surmise that in the experimental infection here studied the greatly reduced therapeutic activity of penicillin in the older infections is due to the fact that the organisms are no longer metabolizing as actively in the inflammatory focus.* When a small inoculum is introduced into a muscle, it at first multiplies rapidly, with an average generation time of forty minutes (cf. Fig. 1), somewhat greater than that observed in a favorable medium *in vitro*. During this period of rapid multiplication and active metabolism the organisms are highly susceptible to the direct bactericidal action of penicillin. As the organisms continue to multiply, however, they probably exhaust the surrounding tissue of nutrilites faster than these can be supplied by the blood stream; and the inflammatory reaction which ultimately develops, with edema, tissue necrosis, cellular infiltration and capillary thrombosis, would accentuate this by limiting the diffusion of foodstuffs into the focus of infection. The leukocytes in the inflammatory focus may compete with the bacteria for nutrilites, and the local accumulation of metabolic products toxic to the bacteria would further reduce their metabolic activity. Presumably because of these various factors the rate of bacterial multiplication is suddenly and sharply curtailed (cf. Figs. 1 to 5), and thereafter the net number of viable organisms remains essentially unchanged over a long period. Simultaneously, the organisms become relatively resistant to the bactericidal action of penicillin.

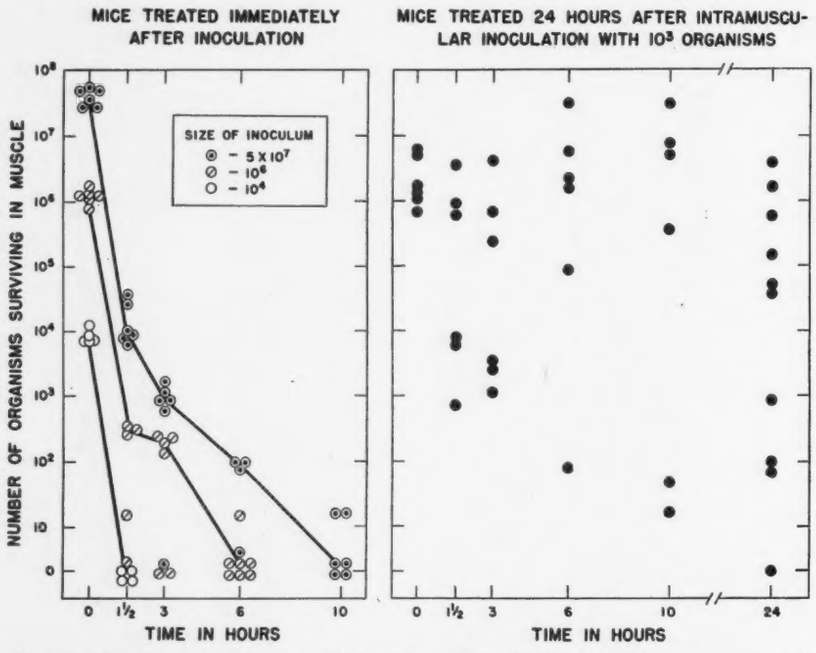

FIG. 3. Showing that the retarded action of penicillin on older streptococcal infections in mice is not due primarily to the larger number of organisms then present. Groups of animals were inoculated into the thigh muscle with 10^4, 10^6 and 5×10^7 organisms, and were then immediately treated with 0.15 cc. of procaine penicillin in oil and monostearate at 10,000 units/cc. In each group the number of organisms surviving in the muscle was determined one and one-half, three, six and ten hours after treatment. The rapid bactericidal effect uniformly observed in these animals (open circles on left side of figure), no matter how large the inoculum, is to be contrasted with the slow and irregular response observed in animals inoculated with 10^3 organisms but allowed to go for twenty-four hours before treatment (solid circles on right side of figure).

If this is the correct explanation for the refractoriness of the bacteria in the established

* The possibility that the inflammatory exudate as such may be inactivating the penicillin locally has not been wholly excluded by the experimental data here reported. However, this would fail to explain the fact that the susceptibility to penicillin decreases abruptly, coincident with an equally abrupt decrease in the net rate of multiplication, and the fact that the established infection is just as refractory to enormous concentrations of penicillin as it is to relatively small concentrations. (cf. Fig. 4.)

infection, the larger the inoculum the shorter should be the time during which the bacteria can continue to metabolize and multiply normally, before the focus becomes an unfavorable medium for growth, and the shorter also should be the time for which penicillin continues to exert a rapid bactericidal effect. This is shown by the experiment of Table 1 and Figure 5. Groups of mice were treated at varying times after inoculation with 50,000,000 bacteria. In the mice treated one and one-half hours after inoculation the bactericidal action of penicillin proceeded at essentially the same rate as in those treated immediately after inoculation. By the third hour of the infection, however, the bactericidal action of penicillin was strikingly retarded; and in mice treated six and nine hours after infection a single injection of procaine

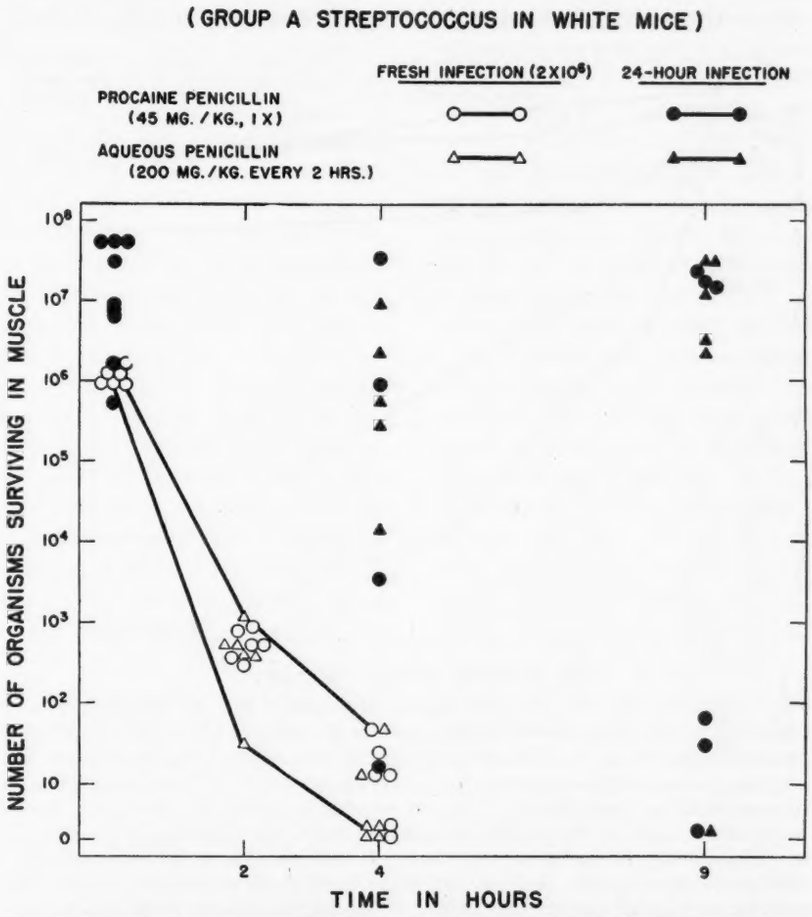

FIG. 4. Showing that the retarded bactericidal action of penicillin in twenty-four-hour streptococcal infections in mice is not due to the inadequate dosage of drug or its limited penetration into the focus of infection. Groups of animals were inoculated with 2×10^6 organisms and treated immediately with a single injection of 0.15 cc. of procaine penicillin at 10,000 units/cc. (open circles in figure) or with 0.16 cc. of sodium penicillin in aqueous solution at 25 mg./cc., the latter dosage repeated every two hours. The procaine penicillin provided an initial average serum concentration of approximately 3 μg./cc., falling to approximately 0.3 μg./cc. after eight hours. The aqueous penicillin provided an average serum concentration after fifteen minutes of 200 μg./cc., and this had fallen to an average of 2 μg./cc. two hours later, when the treatment was repeated. It is evident in the figure that despite the wide disparity in dosages and in the serum concentrations afforded, both forms of treatment effected the same rapid bactericidal action in the freshly inoculated muscle (o-o and △-△), and effected essentially the same slow and irregular bactericidal effect in animals treated only twenty-four hours after inoculation (●-● and ▲-▲).

penicillin produced no significant effect on the number of viable organisms. This rapidly developing retardation of penicillin action was further evidenced in the decreased therapeutic effect of the drug. The single injection of procaine penicillin effectively cured all of twenty-eight animals treated immediately after inoculation, and 27/28 of those treated one and one-half hours after incubation; but in those treated three, six and nine hours after inoculation only 0/28, 1/28 and 0/28 survived.

In summary, with an inoculum of 1,000 bacteria, penicillin was fully effective after six hours and relatively ineffective only after twelve hours, when the median number of viable organisms in the muscle had increased to a median level of 6,000,000 (Fig. 1); but with an inoculum of 50,000,000 organisms the therapeutic activity of penicillin was sharply and abruptly curtailed within three hours (Fig. 5), presumably because the inflammatory focus rapidly became an unfavorable milieu for bacterial growth and multiplication.

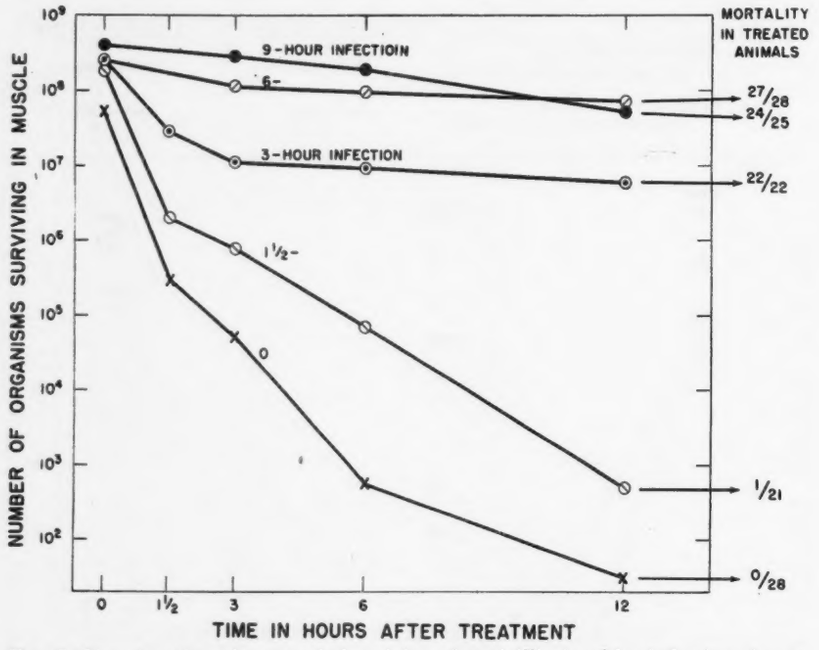

FIG. 5. Showing that the retarded activity of penicillin in older infections is not due solely to the large number of organisms in the muscle. Mice were inoculated intramuscularly with 5×10^7 streptococci. At zero, one and one-half, three, six and nine hours after inoculation, aliquot groups were treated with a single injection of procaine penicillin (0.15 cc. of suspension at 10,000 units/cc.). Each point in the figure is the median number of organisms surviving in the muscle in experimental groups of six mice each. In the animals treated immediately after inoculation, or one and one-half hours later, there was no essential difference in the rate of bactericidal action, or in the therapeutic response. By the third hour the effect of penicillin was strikingly reduced, this despite the fact that there had not been a significant increase in the number of viable organisms; and in animals treated six or nine hours after inoculation, penicillin had essentially no immediate bactericidal effect and all the animals died.

COMMENTS

It has here been shown that when group A streptococci are inoculated into a mouse muscle, the organisms at first multiply rapidly until the number of bacteria in the muscle reaches a plateau value, which then does not change significantly for a period of twelve to twenty-four hours. If treatment with penicillin is initiated during the initial phase of active multiplication, the organisms prove just as susceptible to the drug as they are *in vitro* and sterilization and cure are effected within a few hours. However, if the mice are treated only after the bacterial population in the muscle has stabilized, the organisms are found to have become highly refractory to the drug. Even high concentrations of penicillin then effect only an irregular and slow bactericidal action, and treatment must be

continued for four to six days in order to obtain sterilization and cure.

A number of possible explanations have been considered and tested experimentally (cf. pp. 391–396). The explanation most consistent with experimental results is that in the established infection the inflammatory focus has become a less favorable medium for bacterial growth, either because of an inadequate supply of nutrilites, because of the local accumulation of products toxic to the bacteria, or both. Since penicillin is known to affect only actively growing cells and to be ineffective on "resting" cells, the organisms probably become less susceptible to its bactericidal effect for the very reason that they are growing and multiplying only slowly in an unfavorable environment.

In an important paper on the mode of action of penicillin Bigger[31] reported that penicillin may fail to sterilize large populations of staphylococci *in vitro* because of the presence of "persisters," i.e., organisms which are dormant (nondividing) and for which penicillin is therefore bacteriostatic and only slowly bactericidal, if at all. Such "persisters" were estimated to be few in number and only rarely to exceed one per million bacteria. It was suggested that in some cases failure to cure staphylococcal infections in man with penicillin might be due to the presence of similar "persisters" *in vivo.* In a similar connection Chain and Duthie[30] stated, "Since penicillin kills bacteria only under conditions favourable to growth, it is to be expected that substances inhibiting bacterial growth will antagonize the bactericidal and bacteriolytic effect of penicillin. . . . It is both possible and probable that conditions occur in the body under which staphylococci (and other bacteria) may be in the resting state and thus be insusceptible to the action of penicillin. It is therefore important to give close attention to factors which may bring about these conditions (disinfectants, substances in dead tissues, substances produced by other bacteria, etc.)." The difference between the points of view expressed by Bigger and by Chain and Duthie is quantitative rather than qualitative. Bigger postulated the presence of rare "persisters," so that penicillin would just fall short of effecting total sterilization; while Chain and Duthie suggested the possibility that a large proportion of the bacterial population in a focus not favorable for growth may prove refractory to penicillin. The present data appear to bear out the latter thesis.

Penicillin may thus be relatively ineffective in the experimental infections here studied, for the same reason that it is relatively ineffective when added to a fully grown test tube culture in which the number of viable organisms changes only slowly and in which the slow rate of multiplication is essentially equal to the rate of death. In both cases it is an open question whether the decreased rate of multiplication is due primarily to the exhaustion of the medium (inadequate diffusion of metabolites into the muscle area) or to the release of toxic products into the environment. In either event, the bactericidal action of penicillin is paradoxically reduced because the organisms are not metabolizing and multiplying at their optimal rate.

As will be shown in a later paper,[31a] similar results have been obtained in an experimental endocarditis and nephritis produced with Streptococcus fecalis in rats. It is possible that in man, also, the organisms may be highly refractory to the therapeutic action of penicillin in a focus in which bacteria are not actively metabolizing. This factor may contribute to the paradoxical treatment failures sometimes observed with this drug. It may be noted in this connection that although Leptospira icterohemorrhagiae is inhibited *in vitro* by as little as 0.11 units/ml. of penicillin,[17] and although the drug is said by some workers to be effective in the treatment of early cases all are agreed that penicillin is relatively ineffective[18,32–35] if treatment is delayed until the liver has been seriously involved, as evidenced by the appearance of jaundice. In syphilis, also, penicillin treatment gives the highest percentage of cure in cases treated early in the infection in the seronegative primary stage, but the incidence of treatment failure increases significantly if treatment is delayed until the secondary lesions have appeared.[12–16] In both these situations the decreased therapeutic efficacy of the drug may well be related to the fact that inflammatory foci have been established in which the organisms are no longer growing and multiplying at a maximal rate. Indeed, whenever an inflammatory focus supplies suboptimal conditions for growth, one may anticipate a slower bactericidal action than is observed under optimal conditions *in vitro;* and it may require a longer time period to effect cure with penicillin than would be necessary to kill the corresponding number of organisms in the test tube. The degree to which the foregoing considerations may be affected by the

development of antibodies in the older infections remains to be determined.

One would not anticipate that the refractory state of the bacteria would be overcome by an increase in dosage since the concentration of penicillin is not the limiting factor but rather the physiologic state of the organisms. A more promising approach to successful therapy would appear to lie in prolongation of treatment. In the present experiments, although the bactericidal action of the drug in the established foci of infection was strikingly retarded, it was not wholly abolished and treatment adequately prolonged eventually did effect cure. (cf. Table I.) The possibility of using other antibiotics in combination with penicillin has been discussed by a number of workers. Their finding that in e.g., the treatment of bacterial endocarditis, streptomycin so used is particularly valuable[36–40] is in keeping with the fact that, unlike penicillin, this antibiotic is said to be effective even against organisms which are not actively metabolizing.

SUMMARY

In an experimental infection of mice with group A beta-hemolytic streptococci the organisms at the site of inoculation remained highly sensitive to the bactericidal action of the drug only as long as they continued to multiply at a rapid rate. When the bacterial population in the muscle focus approached a plateau value, they simultaneously became refractory to the drug and it required days to effect sterilization and cure, instead of as many hours. The refractory state of the organisms was not due simply to the large number of organisms present in the older infection, and there was no evidence that a systemic host factor had been impaired. Further, massive doses of aqueous penicillin which provided concentrations in the muscle 10,000 times greater than the minimal effective concentration were no more effective than the relatively low concentrations afforded by an injection of procaine penicillin in oil gelled with aluminum monostearate.

The proposed explanation for the refractory state of the organisms in the older infection is that in such foci the organisms were no longer multiplying and metabolizing as actively as in the fresh infection, due either to the inadequate diffusion of metabolites into the area of infection or to the local release of toxic products. Possible implications with respect of the therapeutic use of penicillin in man are discussed in the text.

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A Long-term Survey of Rheumatic and Non-rheumatic Families*

With Particular Reference to Environment and Heredity

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APPLYING methods devised by Opie and McPhedran[1] for the clinical epidemiologic study of tuberculosis, in which the family was the unit of study, Paul and Salinger in 1931[2] and again in 1934[3] reported on the spread of rheumatic fever in a group of families followed by them between 1929 and 1934. Since that time this familial or domiciliary epidemiologic investigation of rheumatic fever was enlarged to include more rheumatic families and a group of non-rheumatic families for comparison. The survey was developed by periodic visits to the home, examination of all members of the families and observation of their environment over a long period of time in an attempt to determine the factors which might affect the familial prevalence of the disease. A report of this group of families up to 1940 was published by Paul.[4,5]

The present study is the result of a re-examination in their homes of 776 individuals comprising forty rheumatic and thirty non-rheumatic families. The data analyzed were accumulated over a ten- to twenty-year observation period which extended throughout and after the susceptible age of rheumatic fever and involved more than one generation in the families. The object of this follow-up study is to determine whether, after a longer period of observation of the families, the conclusions of the previous reports[4,5] regarding the frequency and nature of the rheumatic episodes and the spread of rheumatic fever within the families still hold and to compare further the rheumatic and control families with respect to heredity and certain environmental factors which might be responsible for the familial prevalence of rheumatic fever.

METHODS

Selection of Families. The rheumatic families studied, at one time numbering 122, were originally selected over a period of years from 1929 to 1939. The criteria on which the rheumatic families were chosen at that time were (1) that one of the members had or had had rheumatic fever and (2) was either on the pediatric or medical ward or enrolled in the dispensary of the New Haven Hospital. Only forty of the original 122 rheumatic families were available for re-examination in 1947 to 1949.

The so-called control or non-rheumatic families were selected during the period of 1930 to 1933 on the basis that it was not known at the time of selection that any sibling of the contact case had rheumatic fever. The contact case was a non-rheumatic child registered in the general pediatric clinic or one of the pediatric specialty clinics of the New Haven Hospital dispensary. Because only twenty-one of the original thirty-five control families could be found or remained willing to submit to examination, their number was increased to thirty by the addition of a comparable group of families who had been observed for a similar length of time. These additional families had originally been selected[3] because one of the members attending the general pediatric clinic or on the pediatric ward was known to have had scarlet fever which was not followed with an attack of rheumatic fever or carditis.

The control families came from essentially the same location and urban or semi-urban environment (i.e., from New Haven County) as the rheumatic group. Both groups of families

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utilized the New Haven Hospital and its outpatient clinics for most of their trivial as well as serious illnesses.

Each family had been visited at home at periodic intervals when additional data were obtained and a physical examination of all members of the household was performed. Since the majority of the group were chosen between the years 1929 to 1939, all had been visited and examined in their homes at least twice and many of them three and four times before the present visit. Data were thus obtained by actual examination of individuals of more than one generation, the index cases (i.e., the individuals who were the basis of selection of the original family groups) and their siblings, their parents and eventually their offspring.

In this manner the study included one group of families with an originally high prevalence of rheumatic fever and another control group relatively free of the illness. It has been designed to compare the frequency and nature of the rheumatic episodes and events which might occur in both groups over a long period of time which would indicate factors responsible for the occurrence of the disease.

Methods of Recent Survey. Preliminary visit: During the follow-up period from November, 1947, to May, 1949, the primary contact with the family was made by the social worker through visits to the home. Members of the family were interviewed to interpret the purpose of the study and to gain consent for examination of all members.

Survey visit: The physician, accompanied by the social worker, visited the home and a medical history was obtained from each member of the household. In most cases the medical histories were in the nature of interim histories. Attempts were made, however, to verify factors which related to rheumatic fever. Additional information regarding relatives with rheumatic fever, causes of death of parents and siblings of the fathers and mothers of the families, sleeping arrangements, rentals and conditions in every house the family had occupied was also obtained. It is important to emphasize that much supplementary information was available in the majority of the individuals examined since they had clinic and hospital records, were known to Social Agencies in New Haven, and had previously been questioned and examined in their homes by physicians of the New Haven Hospital. To supplement our own records information was also obtained from other hospitals, from family physicians and from death certificates.

All available members (in most families this included all members of the family) were examined at the time of the recent visit. The physical examination for the most part was limited to the general state of the individual and a complete cardiac examination including the measurement of blood pressure.

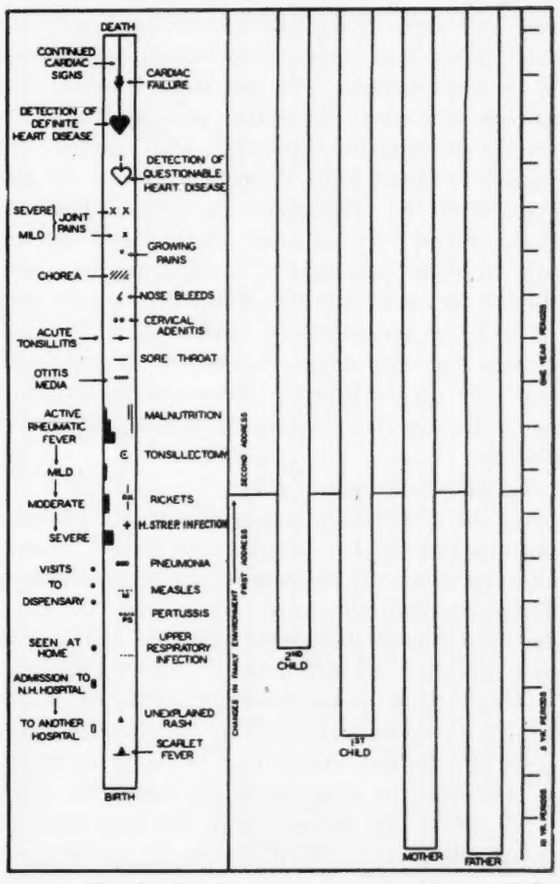

FIG. 1. The family chart and representative symbols.

Family Chart. A compact diagram for recording data related to each individual and the family was utilized in order to visualize events and illnesses of the group as a whole and to determine the relationships of events. The chart used was the one previously used in these family studies.[2–5] The principle of the chart and its legends, for recording various pertinent events, is illustrated. (Fig. 1.) In addition to the family chart, physical examination, medical and social historical findings for each individual were recorded and coded in such a manner that information could be more easily classified and analyzed.

Criteria for Diagnosis of Rheumatic Fever and Rheumatic Heart Disease. The diagnosis of rheumatic fever, in the majority of cases found in this study, had been made by physicians of the University Service of the Grace-New Haven Community Hospital or dispensary at the time of an acute episode. When the individual had not been seen by a physician and the personal story alone had to be relied upon, a diagnosis of rheumatic fever was not made without an unequivocal story of acute polyarthritis with fever, necessitating bed rest and leaving the patient without joint deformity. The only exception to the labeling of an episode as rheumatic fever without such a clear-cut history was in those instances of known scarlet fever during or immediately following which the patient had symptoms or cardiac murmurs suggestive but not diagnostic of rheumatic fever and who on subsequent follow-up examination had characteristic signs of rheumatic heart disease.

No clear-cut history of acute rheumatic fever could be elicited in some individuals who on examination had murmurs and heart sounds characteristic of rheumatic valvular disease. These individuals were considered to have rheumatic heart disease and in some, symptoms such as recurrent sore throats, fatigue, epistaxis, growing pains in childhood were elicited or are known to have occurred but no definite diagnosis of rheumatic fever was or could be made at the time. In some cases diagnosed as rheumatic heart disease no symptoms suggestive of rheumatic fever were elicited or known to have occurred.

No individual was considered to have rheumatic heart disease who did not fulfill the criteria, in regard to clinical cardiac examination, of the New York and American Heart Associations[6] except that clinical evidence of cardiac enlargement (x-rays were not done routinely at this time) was not considered essential for a diagnosis of rheumatic valvular disease. Murmurs heard which did not fit the criteria for a specific valvular lesion were labeled as unexplained murmurs. If there had been a known episode of rheumatic fever and these unexplained murmurs heard, the diagnosis was recorded as possible and potential rheumatic heart disease; but these individuals have not been included with those having rheumatic heart disease. It should be emphasized here that when tl ere was any doubt as to the cardiac signs and a further examination was not feasible, the individuals were not included in the rheumatic group.

In the statistical analyses of the data "t" and "chi square" tests were used to determine the significance of differences in all the comparisons made. The terms significant difference and statistical significance imply the use of one of these tests.

TABLE I

NO. OF INDIVIDUALS EXAMINED AND MEAN AGE OF EACH GENERATION

Generation	Rheumatic Families		Control Families	
	No. of Individuals	Mean Age (yr.)	No. of Individuals	Mean Age (yr.)
1st	79	54.4	53	50.1
2nd	206	23.9	161	21.2
2nd In-laws	53	35.9	32	28.5
3rd	117*	6.4	72	5.1
4th	3			
Total	458		318	

*One individual is a 3rd generation in-law.

Composition of Family Groups. A total of 776 individuals were interviewed and examined; of these 458 belonged to the forty rheumatic families and 318 to the thirty control families. All of the families except two had increased in size since the time of the first examinations ten to twenty years ago. The control and rheumatic families consisted essentially of similar sized families with a similar distribution of large and small families.

For purposes of comparison the family groups were divided into generations. The parents of the original family group are referred to as the *first* generation; the children of these parents, who comprise the index cases and all their siblings, are the *second* generation; the *third* generation are the offspring of these children; and the in-laws are the spouses of the second (and in one instance of the third) generation. The number of individuals in each generation of the rheumatic and control families is given in Table I.

The mean ages of the individuals in the rheumatic and control groups (Table I) as well as the age range and frequency distribution of ages (Fig. 2) for the first three generations were similar, and there were no significant differences between the mean ages of the same generations in either group of families except that the mean

age of the second generation in-laws of the rheumatic families was slightly older than the in-laws of the controls. The sex and race distribution of the individuals examined are given in Table II. The difference in percentage of Negroes in the two groups of families is not statistically significant.

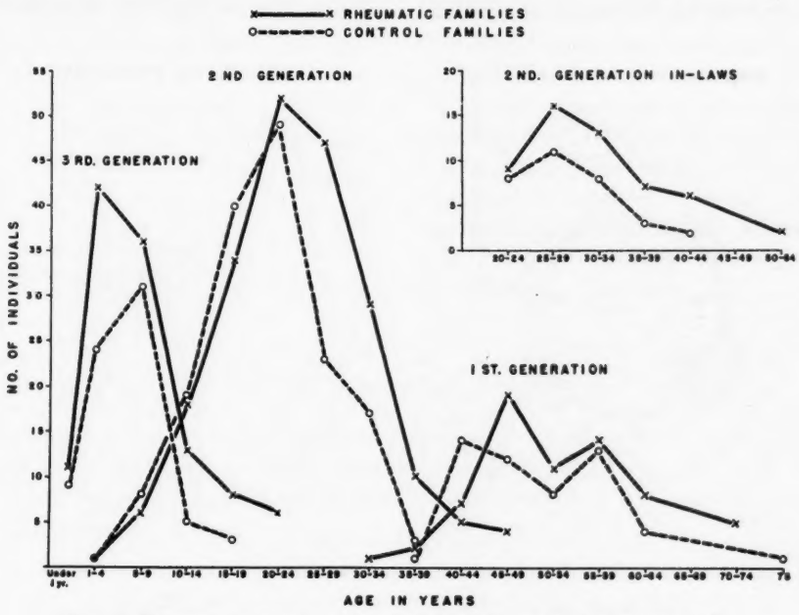

FIG. 2. Frequency distribution of ages of all individuals examined.

RESULTS

Rheumatic History of the Survey Families during the Follow-up Period. Originally within the forty rheumatic families there were seventy-eight cases of rheumatic fever and/or rheumatic heart disease with sixty-eight of these concentrated in the second generation children. In the control families, at the beginning of the study, there were no rheumatic cases among the second generation children but there were three cases in the first generation (i.e., parents). Figure 3 illustrates, at the time of selection and at the present time, each rheumatic individual by family and generation, as well as showing the position in the family of the individual cases and the order of progression of the disease within each family.

The prevalence of rheumatic fever and/or rheumatic heart disease at the present time for each generation is shown in Table III. The rates for the second generation, both rheumatics and controls, are corrected by exclusion of the index cases, since the former group would otherwise be weighted by forty known rheumatics and the controls by thirty known non-rheumatics. Even with exclusion of the known rheumatic index cases there is found to be a very high incidence of the disease in the second generation of the rheumatic families and the difference between these individuals and the same generation controls is statistically significant. Contrary to previous findings in these families, and to the findings in other family studies, the difference in the prevalence of rheumatic fever and/or rheumatic heart disease between the rheumatic

TABLE II

SEX AND RACE OF INDIVIDUALS EXAMINED

	White No.	%	Negro No.	%	Oriental No.	%	Total
Rheumatic Family Individuals							
Male	202	86.7	31	13.3	--	--	233
Female	198	88.0	27	12.0	--	--	225
Total	400	87.3	58	12.7	--	--	458
Control Family Individuals							
Male	153	91.6	14	8.4	--	--	167
Female	137	90.7	13	8.6	1	0.7	151
Total	290	91.2	27	8.5	1	0.3	318

and control families in the first generation is not statistically significant. The differences between the two groups of families in the in-laws and third generation are likewise not significant. It should be pointed out that the individuals of the third generation as a group are as yet too young to have passed through the peak years for the occurrence of rheumatic fever. Another decade must elapse before the true incidence of the disease in this generation can be determined.

The majority of all the rheumatic individuals in the first and second generations of both groups of families were known to have had acute rheumatic fever. Table IV gives the incidence in this group* of rheumatic fever and of rheumatic valvular heart disease diagnosed at the time of or subsequent to the initial attack. It is seen from this table that in only 15.7 per cent of the cases was a diagnosis of rheumatic heart disease made without a history of acute rheumatic fever. Of the eighty-six individuals with rheumatic fever, 28 per cent have not shown evidence of valvular heart disease while an equal percentage

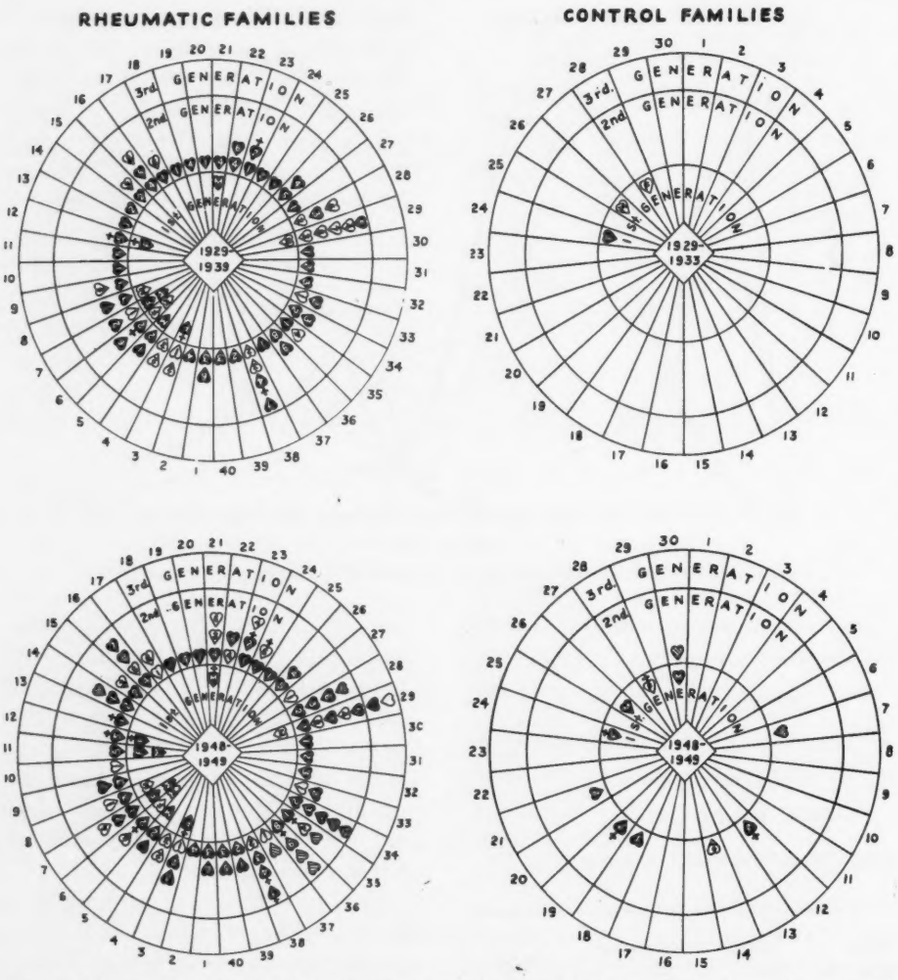

FIG. 3. Rheumatic individuals in rheumatic and control families. Open heart is a case of known acute rheumatic fever without heart disease. Black heart is a case of rheumatic heart disease with or without known acute rheumatic fever. Cross-hatched heart is a second generation in-law with rheumatic fever and/or rheumatic heart disease. F, father of the family; M, mother of the family. Numbers within each heart designate position in the family, i.e., 1 is the firstborn, etc. The cases are given from the center outward in the order in which they occurred in the multiple case families. + means deceased.

* Only the first and second generations are included in this particular analysis because they are the group that have been observed throughout the survey period whereas the third generation and second generation in-laws have been examined only once.

TABLE III

PREVALENCE OF RHEUMATIC FEVER AND/OR RHEUMATIC HEART DISEASE

Generation	Rheumatic Families			Control Families		
	No. of Individuals	R.F./R.H.D.† No.	%	No. of Individuals	R.F./R.H.D. No.	%
1st	79	12	15.2	53	4	7.5
2nd*	166	41	24.7	131	5	3.7
3rd	116	2	1.7	72	0	0.0
2nd In-laws	53	6	11.3	32	2	6.2

*Corrected for index cases (40 rheumatics and 30 controls omitted).
†Rheumatic fever and/or rheumatic heart disease.

the families, giving a rate for new cases of 9.3 per cent. On the other hand, five new cases appeared among the 161 persons of the same generation control group, a rate of 3.1 per cent. From these figures it is evident that during the study years three times as many second generation children in the rheumatic families developed rheumatic fever as compared with the controls, indicating that the factors responsible for this significant difference were probably continuous during the study period. The new cases in the rheumatic families occurred with equal frequency in those families which originally had a single case as in those with more than one case at the time of selection.

TABLE IV

INDIVIDUALS WITH RHEUMATIC FEVER AND/OR RHEUMATIC HEART DISEASE

	R.F.	R.F. + R.H.D.	R.F.→R.H.D.	R.H.D.	Totals
1st generation individuals of:					
Rheumatic families	4	1	4	3	12
Control families	1	1	0	2	4
2nd generation individuals of:					
Rheumatic families	17	21	33	10	81
Control families	2	2	0	1*	5
Totals	24	25	37	16	102
Percentage of those with R.F.	27.91	29.07	43.02	15.7†	

R.F. = Acute rheumatic fever without valvular heart disease.
R.F. + R.H.D. = Acute rheumatic fever plus rheumatic heart disease first diagnosed at time of acute episode.
R.F.→R.H.D. = Acute rheumatic fever. Rheumatic heart disease first diagnosed after the acute episode.
R.H.D. = Rheumatic heart disease without a history of acute rheumatic fever.

*Diagnosed seven years after scarlet fever.
†Percentage of total (102) rheumatics.

had such evidence at the time of the initial acute attack.

For computing the incidence of new cases the second generation was focused on, since this was the group of individuals who had passed through the most vulnerable age (five to fifteen years) for rheumatic fever during the study period from 1929 to 1949. By 1948 to 1949, thirteen new cases of rheumatic fever and/or rheumatic heart disease had occurred in the 138 individuals of the second generation rheumatic families who were non-rheumatic at the time of selection of

Spread of Rheumatic Fever through the Family. Rheumatic fever in a family, as has been previously noted by Paul,[4,5] usually appears first in a senior child and later in a junior sibling, and the interval between the two cases is a matter of years rather than days or weeks as is found in most of the common contagious diseases. This oblique manner of spread of rheumatic fever is illustrated in Figure 4, a segment of the L. family chart.

In the twenty-six families with multiple cases of rheumatic fever in the second generation this

oblique manner of spread was demonstrated repeatedly and in regard to the progression of cases within the family it was found that 70.7 per cent of the cases of rheumatic fever occurred first in a child senior in position in the family to the sibling who next acquired the disease.

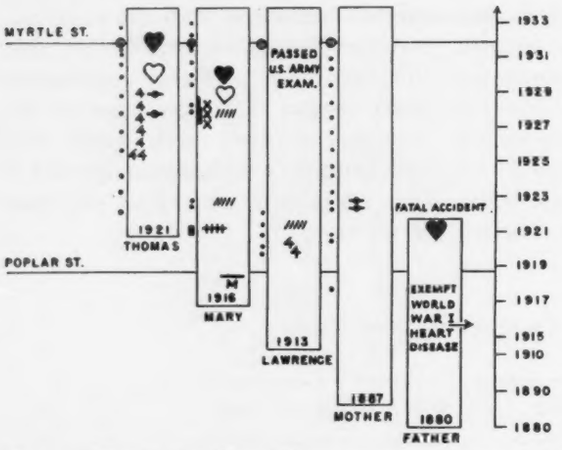

FIG. 4. The L. family; oblique manner of spread of rheumatic fever. (Symbols as in Fig. 1.)

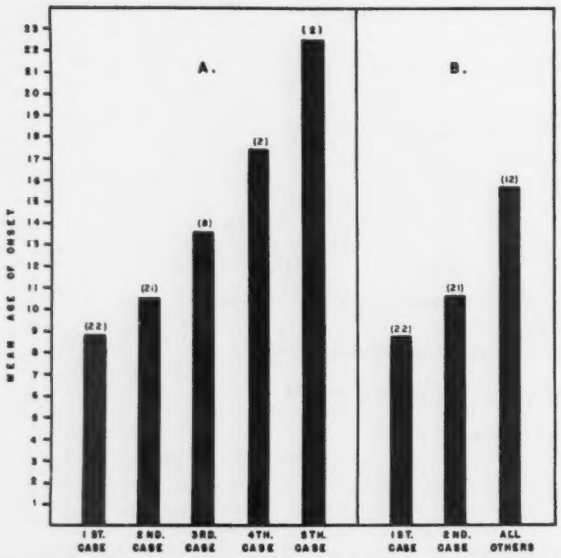

FIG. 5. Mean age of onset of rheumatic fever in multiple case families. A, onset of successive cases; B, onset of first, second and all other cases. ( ) stands for number of individuals.

In the rheumatic family group the range of age at onset of rheumatic fever in the second generation was from three to thirty-three years and the majority of cases occurred within the usual age of greatest susceptibility to the disease of five to fifteen years. The mean age of onset for the group was 10.4 years. For rheumatic fever cases in the control families the age range at onset was from seven to fourteen years and the mean age 11.2 years. The average age of onset in the single case rheumatic families (known in twelve of the fourteen cases) was 7.9 years, not significantly different from the first cases in the multiple case families.

In considering the average age of onset for the first and subsequent cases within the multiple case families, the onset of rheumatic fever was known in fifty-five of the sixty-seven cases. In these cases there was a definite trend toward increasing age of onset, from a mean of 8.9 years for the first case to 22.5 years for the fifth. (Fig. 5A.) Because the number of individuals in the third, fourth and fifth case groups was small, for purposes of statistical analysis they were treated as a single group whose mean age was 15.8 years. (Fig. 5B.) Comparing the first case with the second, and the latter with the subsequent cases, no significant difference is found. But a comparison of the mean age for the first case (8.9 years) with the mean age for all subsequent cases (12.5 years) or with the mean for the third, fourth and fifth (15.8 years) is statistically significant. Thus in the majority of instances, although the first case to occur within a family was the senior of the next case in relation to position in the family, he was younger in years when he developed rheumatic fever than the siblings who subsequently acquired the disease and there was a definite trend toward increasing age of onset from the first to the fifth case within a family.

Illnesses Preceding the Onset of Rheumatic Fever in Rheumatic and Control Families. In both the rheumatic and control families combined (excluding those who married into the second generation) there were 104 cases of rheumatic fever and/or rheumatic heart disease. In sixty-three of these cases sufficient data were recorded about illnesses directly preceding the onset of rheumatic fever to indicate the nature of these illnesses. (Table v.)

In many families epidemics of upper respiratory infections due to beta-hemolytic streptococci existed within the family for months and were followed with the appearance of rheumatic fever in one or more members of the family. A good example of this situation may be seen in Figure 6 representing the R. family. Repeated waves of tonsillitis, otitis media, cervical adenitis and sore throat due to beta-hemolytic streptococci occurred over a period of years in all the

children and was followed with rheumatic fever in one of them.

Although an exception to the usual oblique spread of rheumatic fever, the P. family in which several cases of rheumatic fever followed an epidemic of scarlet fever again illustrates the occurrence of streptococcal disease preceding the onset of rheumatic fever. (Fig. 7.)

TABLE V

NATURE OF ILLNESSES IMMEDIATELY PRECEDING ONSET OF RHEUMATIC FEVER

Diagnosis	No. of Cases
Sore throat	21
Tonsillitis	11
Scarlet fever	10
Common cold	7
Otitis media	5
Cervical adenitis	4
Sore throat proven to be due to beta-hemolytic streptococcus	4
Sinusitis	1

Social and Economic Conditions in the Families. Poor living conditions have been thought to play an important role in the epidemiology of rheumatic fever in England[7–9] and in the United States.[10–13] If this is true, it would be expected that the group of rheumatic families came from poorer environmental circumstances than did the control families. With the knowledge that all factors in the environment could not be weighed, a few were selected for investigation in some detail, namely, economic status, housing, family size and crowding. It was thought that these features of the environment would be good indicators of the family's general living pattern.

Economic levels: Both groups of families, having been chosen from the wards and outpatient clinics of the New Haven Hospital, were made up essentially of low income families. To gauge income level in the past the family was questioned in detail about their former economic status, information was secured from social service records in the clinic and hospital charts and from records of other social agencies in the community.* These data were obtained for the years 1930 to 1931 at which time the peak incidence of rheumatic fever cases was reached among the original families and closely approximates the time of selection of the majority of families. From these data the family's past income was graded according to family budget standards for 1930 to 1931,† as low, adequate or comfortable, as given later.

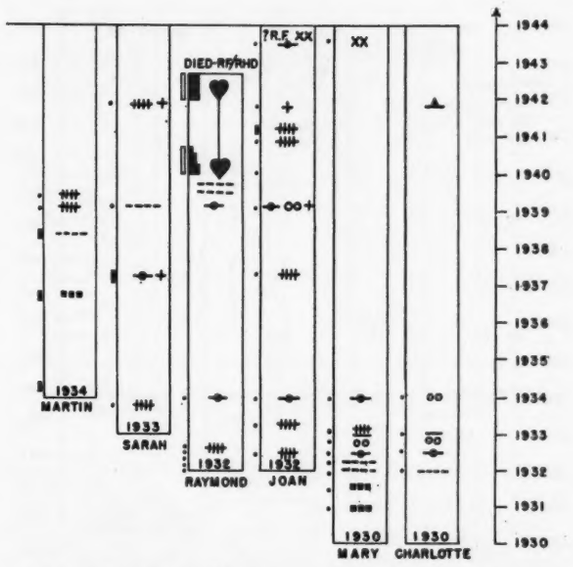

FIG. 6. The R. family; an example of repeated upper respiratory infections in members of the family followed with rheumatic fever. (Symbols as in Fig. 1.)

It was possible to secure more accurate information of the family's present (1948 to 1949) income level by questioning the current wage earners and these data were graded according to 1948 to 1949 family budget standards.† Low incomes were those judged to be too meager to cover adequate nutrition, proper clothing and other basic essentials of family life; the adequate incomes were those which made possible a minimum yet acceptable standard of living without luxuries but would not cover any unforeseen expenses; the comfortable incomes were those large enough to provide an ample budget for essentials plus some luxuries.

As seen in Table VI the rheumatic and control families did not differ significantly in average family income at the time of the peak incidence

* Visiting Nurse Association of New Haven, the Children's Center, and the Connecticut Division of Child Welfare.

† Supplied by Mrs. Frances Coles, Home Economist for the Visiting Nurse Association of New Haven.

of rheumatic fever in the families and consisted primarily of a low income group. It is evident that in 1948 to 1949, although the economic status of both groups of families had improved remarkably, a third of the controls and slightly but not significantly more of the rheumatic

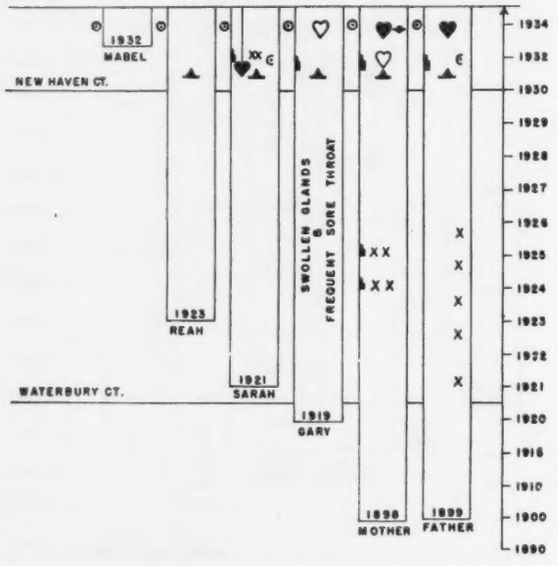

FIG. 7. The P. family; epidemic type of spread of rheumatic fever. (Symbols as in Fig. 1.)

group were still living on less than adequate incomes.

Size of family: A comparison of size was made between the two family groups at the time of selection as well as for 1948 to 1949 and no significant differences were revealed. Both showed the same distribution of large and small families.

Housing: Again the years 1930 to 1931 were chosen for study of housing conditions in the rheumatic and control groups as representing conditions during the peak period for rheumatic activity. The criteria for the grading of housing were those used by the Office of Rent Control, New Haven: (1) Substandard was used to describe those homes without central heating, with shared toilet facilities and in need of major repairs; (2) passable homes were mainly domiciles with central heating, private toilets and the need for major repairs met; and (3) good, where housing was good in respect to construction and repair. The former rent paid by families, their descriptions of past housing and conditions observed and recorded at the time of initial and subsequent visits to the home were the main aids in arriving at these rough classifications. From these data it was estimated that 93.4 per cent of the individuals in the rheumatic families were in 1930 to 1931 living in substandard housing whereas 70.6 per cent of the controls dwelled in

TABLE VI

INCOME LEVEL

Income Level	Rheumatic Families		Control Families	
	No. of Individuals	%	No. of Individuals	%
	1930–1931*			
Low	179	93.7	114	85.9
Adequate	12	6.3	19	14.1
Comfortable	0	0	0	0
	1948–1949			
Low	175	36.7	102	33.2
Adequate	235	52.3	172	56.0
Comfortable	39	11.0	33	10.8

*The years of peak incidence of rheumatic fever.

TABLE VII

HOUSING

Type of Housing	Rheumatic Families (%)	Control Families (%)
	1930–1931*	
Substandard	93.4	70.6
Passable	4.5	26.3
Good	2.1	3.1
	1948–1949	
Substandard	57.7	50.4
Passable	22.1	28.1
Good	20.2	21.5

*The years of peak incidence of rheumatic fever.

similar housing facilities. (Table VII.) This is a highly significant difference and reflects the predominantly poorer living circumstances of the rheumatic families in spite of the fact that

there was no difference in income level and family size.

During the study period there was general improvement in housing for all families studied. Table VII shows that now only 7.3 per cent more of the rheumatic families than the controls are living in substandard housing.

TABLE VIII

CROWDING

Crowding In:	Rheumatic Families (%)	Control Families (%)
	1930–1931*	
Home	85.9	76.6
Bedroom	59.8	26.3
Bed	23.4	6.7
	1948–1949	
Home	30.5	24.7
Bedroom	19.8	17.6
Bed	1.5	2.3

*The years of peak incidence of rheumatic fever.

Crowding in the home: It has been thought by many observers[8,12,14] that crowding definitely affects the prevalence of rheumatic fever. Therefore, in this study crowding within the home was considered in detail by means of three measuring aids, namely, the number of persons per room, number of persons per bedroom and number of persons per bed. Crowding was said to exist when there was over one person per room, two persons per bedroom and more than two persons per bed. From the figures in Table VIII it is obvious that the crowding index for all three measurements was significantly higher for the rheumatic families than for the controls during 1930 to 1931, particularly in bedroom and bed. A specific example of crowding in the rheumatic families was that of a family in which fourteen members were living in four rooms. With one room used mainly for kitchen and eating purposes, all fourteen individuals were divided among the other three rooms in six beds for sleeping purposes. There were four rheumatics in this family.

Over the study period crowding in the home diminished considerably in both groups of families but remained slightly greater for the rheumatic families. (Table VIII.)

Heredity in Rheumatic Fever. A high familial prevalence has long been noted[15–17] to be present in rheumatic fever but the mere concentration of a disease within a family does not necessarily indicate that the disease in question is inherited. Some studies[18–20] have shown that parents and close relatives of rheumatic children show a greater percentage of rheumatic fever than the general population or than control families and thus assumed an hereditary factor in the development of rheumatic fever. Wilson and Schweitzer,[21] applying genetic methods to a study of many families, concluded that the susceptibility to rheumatic fever is inherited as a single autosomal recessive gene, and that this and not environment is responsible for the familial prevalence of rheumatic fever.

TABLE IX

HEREDITY IN RHEUMATIC FAMILIES

	No. of Families	Total No. Children (Second Generation)	Rheumatic Children Predicted*	Rheumatic Children Observed No.	Rheumatic Children Observed %
Neither parent rheumatic	31	168	55	59	35.1
One parent rheumatic	6	29	15	16	55.2
Both parents rheumatic	3	9	9	6	66.7

*Predicted on basis of single autosomal recessive gene with correction for family size and bias in selection of families. (See Wilson.[21])

In analyzing the present series of families the method used by Wilson was applied. Briefly, this consists of the use of genetic formulas[21] for prediction of the probable occurrence of a disease in the progeny of a given mating assuming a single recessive Mendelian inheritance. This implies that once rheumatic fever appears in a child, as is the situation with all the rheumatic families in the study, both the parents transmit the recessive gene.

In the present series of rheumatic families it was found (Table IX) that the observed number of cases of rheumatic fever closely approximated the cases predicated on the basis of a single autosomal recessive gene except in the mating of two rheumatic parents. This might indicate that in New Haven penetrance* of the gene may be incomplete in that only 66.7 per cent of all the susceptibles manifested the disease as com-

* The percentage of hereditary susceptible individuals who manifest a trait; in this case, rheumatic fever.

pared with 86 per cent in the New York families studied by Wilson. If this were true, however, one might expect that this incomplete penetrance of the recessive gene would also operate in the other types of matings and result in less than the predicted number of cases, which was not true in our families. The possibility of illegitimacy of the non-rheumatic children in the three families with both parents rheumatic, as an explanation for the discrepancy between the predicted and observed number of rheumatic children in these families, cannot be excluded. However, one non-rheumatic child was found in each family of this group and it seems unlikely that there would be illegitimacy in all the families of any particular group.

The five cases of rheumatic fever and/or rheumatic heart disease in second generation controls occurred in families whose parents were non-rheumatic. In none of the four control families in which a parent was rheumatic are there any children (of a total of twenty-five) who have had rheumatic fever or rheumatic heart disease. Thus in these families the disease occurred in the second generation without apparent relation to the presence or absence of parental rheumatic fever.

The prevalence of rheumatic fever in relatives of the first generation was based on history alone except in a few instances in which the rheumatic relative had a clinic or hospital record. One might expect a history of rheumatic disease more frequently in relatives of the rheumatic families than in relatives of the control group merely on the basis of greater familiarity with the disease and yet it was found that rheumatic fever in a close relative (mother, father, sibling, aunt or uncle) of the first generation was present in only six rheumatic families (15 per cent) as compared with three control families (10 per cent), which is not a significant difference.

Twins. No conclusion can be drawn from the study of twins in the families since there were only two sets of presumed identical twins and only one of the four individuals was rheumatic. Thus one set of twins was similar and the other dissimilar with respect to rheumatic fever.

Diseases Other Than Rheumatic Fever. Of related significance in the present study is the prevalence of non-rheumatic illnesses in the control and rheumatic families. It might be found, for instance, that not only was there more rheumatic fever but that a variety of other diseases were also more prevalent in the rheumatic families, indicating a general susceptibility among these families or that conditions were favorable in their homes for the spread of infections of various types. The incidence of heart disease other than rheumatic (Table x) of several other diseases (Tables xi, xii), and of the common contagious diseases did not differ significantly in the comparable age groups of the rheumatic and control families.

TABLE X

INCIDENCE OF HEART DISEASE OTHER THAN RHEUMATIC HEART DISEASE

Generation	Rheumatic Families			Control Families		
	Total No. Individuals	Individuals with Heart Disease No.	%	Total No. Individuals	Individuals with Heart Disease No.	%
1st	79	26	32.9	53	18	33.9
2nd	206	3	1.5	161	2	1.2
2nd In-laws	53	1	1.9	32	0	0.0
3rd	116	1	0.9	72	1	1.4

TABLE XI

INCIDENCE OF HYPERTENSION

Generation	Rheumatic Families		Control Families	
	No. of Individuals	%	No. of Individuals	%
1st	8	10.1	4	7.5
2nd	3	1.5	3	1.9
2nd In-laws	5	9.4	4	12.5

TABLE XII

INCIDENCE OF VARIOUS DISEASES IN THE SECOND GENERATION

	Rheumatic Families		Control Families	
	No. of Individuals	%	No. of Individuals	%
Glomerulonephritis	3	1.5	6	3.7
Pyelonephritis	3	1.5	1	0.6
Poliomyelitis	1	0.5	5	3.1
Infectious hepatitis	3	1.5	3	1.9

Because of recent experimental evidence[22–24] of the similarity between pathologic lesions in

rheumatic fever and those in animals produced by hypersensitivity to various substances including beta-hemolytic streptococci, an analysis was made of the incidence of allergies in the individuals examined. There was no significant difference in the incidence of hay fever, asthma, serum sickness or food or drug allergies, considered as a group (Table XIII) or separately, between the control and rheumatic families nor was there a difference between the rheumatic individuals and their non-rheumatic siblings in the rheumatic families.

TABLE XIII

INCIDENCE OF ALLERGIES

Generation	Rheumatic Families		Control Families	
	No. of Individuals	%	No. of Individuals	%
1st	14	17.7	6	11.3
2nd				
Rheumatic Individuals	16	19.7		
Non-rheumatic Individuals	31	24.8		
All Individuals	47	22.8	34	21.05
3rd	19	16.4	15	20.8

*Hay fever, asthma, serum sickness, and food and drug allergies.

SUMMARY AND CONCLUSIONS

The present study, involving observation over a ten to twenty year period of a group of forty rheumatic families showing a high prevalence of rheumatic fever, and a group of thirty families in which the disease was relatively rare, revealed certain factors that have probably influenced the prevalence of the disease in the former group.

Poor housing and crowding within the home were found to be significantly greater in the rheumatic families than in the controls. It is believed that the latter condition, allowing for more intimate contact among members of the family group, may be conducive to the spread and maintenance of upper respiratory infections (particularly those due to hemolytic streptococci) within the family circle, and it is this type of repeated infection that may lead to the occurrence of rheumatic fever.

Analysis of the rheumatic families on the basis of parent matings (with suitable corrections for bias in selection and for family size) revealed that the occurrence of rheumatic fever in the children approximated the pattern of a single autosomal recessive gene except in the mating of two parents with the disease. In this mating only 66 per cent of the children in the families had the disease whereas on a genetic basis 100 per cent would be expected. This fact, plus the lack of difference between the prevalence of rheumatic fever in controls and rheumatics of the first generation (the parents in the families) and their close relatives, suggests that heredity, if a factor in the prevalence of rheumatic fever, does not follow a specific genetic pattern.

The data suggest that if heredity plays a role, it is perhaps one of inherited susceptibility, not strictly predictable because of an important controlling factor in the occurrence of the disease, namely, repeated infections due to the beta-hemolytic streptococcus. It might be speculated that the inherited factor may not be an increased susceptibility to rheumatic fever *per se* but rather an altered host response to repeated infection, the latter being enhanced or becoming recurrent within the family circle when poor housing and crowding in the home are present.

Acknowledgment: The authors wish to acknowledge the stimulation, guidance and assistance of Dr. John R. Paul who initiated this study and made possible its continuation. Mr. Harry Auerbach, Assistant Professor of Biostatistics, Department of Public Health, assisted in the statistical analyses of the data.

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Rickettsialpox*

Report of Four Apparent Cases in Pennsylvania

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IN June, 1946, a new illness was observed in the residents of a housing development in the Borough of Queens in New York City. The disease, originally referred to as Kew Gardens' spotted fever,[1] quickly became epidemic in the area and at the same time similar cases were reported from other parts of New York City.[2] The disease was subsequently identified as a new entity caused by a newly discovered species of rickettsia.[3] Because some of the lesions were papulovesicular and confused with varicella the disease was named rickettsialpox and the causative organism was designated R. akari.[4] Epidemiologic and entomologic studies later demonstrated that the primary host of the infection was the common house mouse (Mus musculus) and that the organism was transmitted to man accidentally by a blood-sucking rodent mite (Allodermanyssus sanguineus).[5,6] Since the recognition of the disease in 1946 about 150 cases a year[7] have been reported to the New York City Department of Health. The first published report of a case outside New York City, in a resident of Boston, Massachusetts, recently appeared.[8]

This article is a report of four cases in which the diagnosis of rickettsialpox appears justified on the basis of the clinical and serologic findings. Three of the patients lived in the Richmond section of Philadelphia and were presumably infected at home, while the fourth patient, a pharmaceutical laboratory worker, was apparently infected in his place of employment, located in suburban Delaware County. None of our patients had been in New York City prior to the onset of infection.

CASE REPORTS

CASE I. (Fig. 1.) R. S., a fourteen year old white boy, was well until May 1, 1949, when a sore throat, nausea, vomiting, fever, chill and backache developed. On the following day a rash on the lower extremities appeared which spread rapidly to the torso and face. On May 4th the patient complained of slight stiffness of the neck. The next day he was admitted to the Philadelphia Hospital for Contagious Diseases with the provisional diagnosis of meningitis. He had had measles, pneumonia, pertussis and adenoidotonsillectomy and, in 1945, was immunized against smallpox. He lived with his mother, a winder in a wool mill, five sisters and three brothers, all of whom were well. The family resided in Kensington, an industrial section near Port Richmond, Philadelphia.

Examination on admission to the hospital revealed a well developed, white boy who was acutely ill with a temperature of 103.4°F., pulse 112 and respirations of 36. The significant findings were a mild injection of the conjunctivas, left tympanum and pharynx. Resistance to flexion of the neck was mild and the cervical lymph nodes were enlarged and tender. The spleen and liver were not palpable but tenderness was elicited over the left upper quadrant of the abdomen. Lymphadenopathy was generalized. A discrete, erythematous, papular rash was present over the chest, abdomen, back and extremities, including the palms and soles. There was a similar rash and violescent flush over the face. The admission impressions were meningococcemia, rickettsiosis, infectious mononucleosis or typhoid fever.

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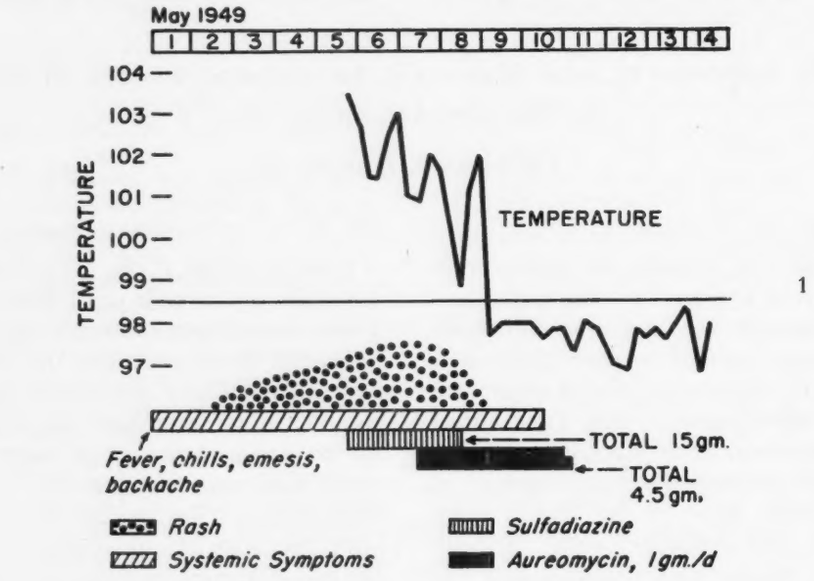
X-ray of the chest was normal. Erythrocyte sedimentation rate (Wintrobe) was 20 mm. in one hour. The red blood count was 4.3 million; white blood count 7,700, with neutrophiles 72 per cent and lymphocytes 28 per cent. Urinalysis was normal. Spinal fluid showed two gens and of the Weil-Felix tests are tabulated and discussed hereinafter.

Sulfadiazine was given from May 6th to 8th. On May 7th the edge of the spleen became palpable and tender, several new lesions appeared on the skin and the patient complained



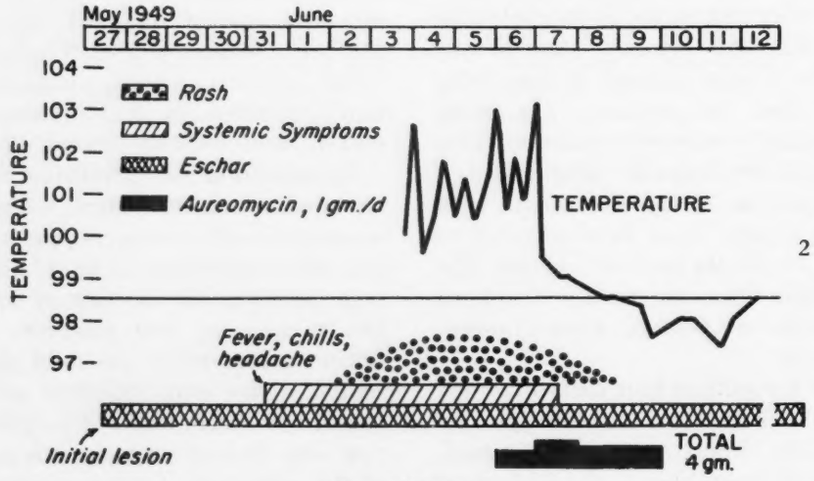


FIG. 1. Case I.
FIG. 2. Case II.

cells, 15 mg. per cent protein; smear was negative and culture showed no growth. Blood Kolmer-Kline and Wassermann tests were negative. On May 6, 1949, a blood culture was sterile. Blood agglutination titers for typhoid and paratyphoid were normal. Heterophile antibodies were absent on May 7th but were present (1:112) on May 13th. Results of all complement fixation tests with rickettsial antifrequently of backache and abdominal pain. Aureomycin was started on May 7th, 250 mg. every four hours for six doses then every six hours for twelve doses. On May 8th the rash began to fade; the spleen, however, was still palpable and tenderness in the left upper quadrant was still present. The temperature became normal within twenty-four hours after aureomycin was started and twelve hours later the

constitutional symptoms began to abate. The patient received a total of 4.5 gm. of aureomycin over four days and was discharged on May 14th.

Comment: The initial lesion in this patient was not apparent. There was no evidence of regional lymphadenitis to suggest that there was an occult lesion in any part of the body. The eruption was erythematous, discrete and papular, without suggestion of vesiculation. The weak Weil-Felix reaction and a progressive rise in the titer of the complement fixation test to antigens of the Rocky Mountain spotted fever-rickettsialpox group strongly suggest the diagnosis of rickettsialpox. (Table I.) It is presumed that the patient's complaints, which began on May 1, 1949, two days before the appearance of the rash, represent the beginning of the febrile period of the secondary rash of rickettsialpox, the primary lesion being unrecognized. The significance of the low titer of heterophile antibodies in the patient's serum is not known.

CASE II. (Fig. 2.) Mrs. M. S., a forty-nine year old white woman, the mother of Case I, was well until May 27, 1949, when a "pimple" developed on the right thigh. She treated it with hot compresses and black salve. On May 31, 1949, the patient had chills, temperature of 100°F., headache, backache, sore throat and vomiting, for which she was given oral penicillin and sulfadiazine. On June 2nd, two days after medication, a rash appeared on the thighs and later on the back, chest, abdomen and arms. On the following day the patient was admitted to the Philadelphia Hospital for Contagious Diseases with the tentative diagnosis of Rocky

TABLE I

SUMMARY OF SEROLOGIC TESTS OF FOUR PATIENTS WITH PROBABLE RICKETTSIALPOX

Patient	Clinical Diagnosis	Day of Illness Blood Drawn	Complement Fixation Results						Weil-Felix‡ Results	
			Routine Soluble Antigens				Washed† Rickettsiae			
			RP*	RMSF	ET	MT	RP	RMFS	OX19	OX2
Case I, R. S.										
S_1	RP	5	<2		<2	<2				
S_2		8	64	8	<2	<2			40	20
S_3		12	512	256	<2	<2	80	<10	40	80
S_5		99	64	64					40	20
S_6		162	32	32					20	20
Case II, M. S.										
S_1	RP	3	<4							
S_2		11	128	128					40	40
S_3		27	64	64			40	<10		
S_4		73	16	4						
S_5		146	<8	<8						
Case III, C. A. R.										
S_1	RP	5	<2		<2	<2				
S_2		16	32						<20	
S_3		32	32		<4	<4				
S_4		39	16		<2		10	0	20	20
S_5		150	4				20	<10	<20	
Case IV, J. S.										
S_1	RMSF or RP	7	<2						<20	
S_2		17	256+						80	20
S_3		34	256+						80	20

* RP = rickettsialpox; RMSF = Rocky Mountain spotted fever; ET = epidemic typhus; MT = murine typhus; S = serum specimen. Figures are the reciprocal of the dilutions.

† Results obtained from Army Medical Department Research and Graduate School.

‡ Highest titer obtained by one of four methods.

Mountain spotted fever or cutaneous anthrax. She has four sons and five daughters and is employed as a winder in a wool mill. She had had pneumonia, measles and pertussis.

Examination revealed a well developed and well nourished white woman in no acute distress. Temperature was 100°F., pulse 100, respirations 24. The significant findings were right otorrhea, markedly injected pharynx, palatal enanthem, apical systolic murmur and an elliptic-shaped lesion on the anterior right thigh. The lesion was a sharply demarcated area of erythema measuring about 2.5 cm. in its long diameter, with a black central eschar. The regional inguinal lymph nodes were enlarged and tender. There was a discrete erythematous maculopapular rash over the back, chest, abdomen and extremities.

The red blood cell count was 4.06 million, white blood cell count 7,300 with 72 per cent neutrophiles and 28 per cent lymphocytes. Urinalysis was normal. Blood urea nitrogen was 11 mg. per cent and blood sugar 98 mg. per cent. Smear and culture of the eschar were negative for anthrax; blood culture was sterile. Heterophile antibodies were present in 1:112 dilution on June 5th but absent on June 27th.

Aureomycin was started on June 6th, three days after admission, on a schedule of 250 mg. every four hours for six doses, then every six hours. A total of 4 gm. was given over three and a half days. The temperature became normal on June 7th after three doses of aureomycin. The headache persisted, however, but no new lesions appeared. In the evening of that day the patient had a bout of profuse perspiration and the headache ceased. The rash faded gradually and was gone by June 9th. The patient was discharged June 12, 1949.

Comment: The initial lesion and regional lymphadenitis in this patient are typical of rickettsialpox as reported by others. Four days after the appearance of the initial lesion constitutional symptoms with fever developed and two days later a generalized papular eruption appeared. The temperature rose as the rash became generalized and declined as it faded over a seven- to eight-day period. The eschar persisted for over three weeks; this is typical of rickettsialpox. Support for the clinical diagnosis of rickettsialpox is found in the persistently low Weil-Felix reaction and a rising titer for complement fixing antibodies for antigens of the Rocky Mountain spotted fever-rickettsialpox group. (Table I.)

This patient's symptoms began twenty-six days after her son became ill, indicating that the disease was probably not contracted from him. The disease in the son was moderately severe and in the mother relatively mild. Since there was no recollection of an insect bite in either case, it was not possible to ascertain the incubation period.

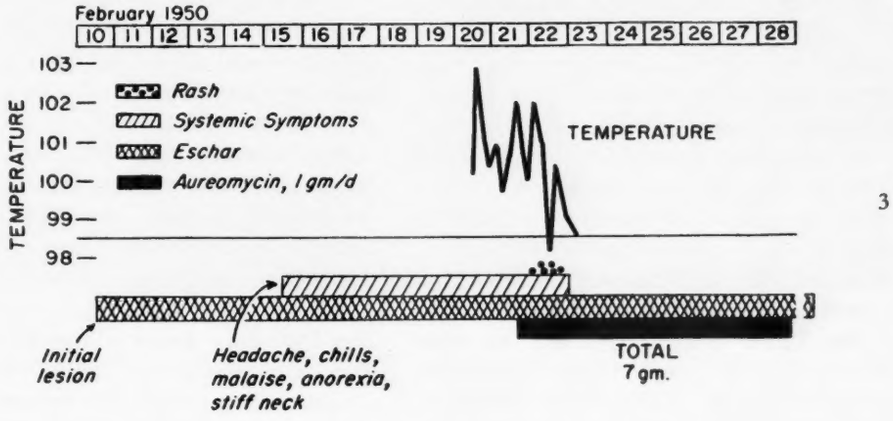
CASE III. (Fig. 3.) C. A. R., a thirty-two year old white man, was admitted on February 20, 1950, to the Woman's Medical College Hospital, with the chief complaint of fever and stiff neck. Chilliness and sleepiness had been noted in mid-afternoon of February 15th. This malaise lasted for two hours and recurred on the two following days. On February 18th stiffness of the neck with pain on lateral motion was noted. Temperature was 100.6°F. On February 19, the day before admission, the patient had a severe chill in the afternoon with a temperature rise to 102°F. Frontal headache, with pain behind the eyes, was moderately severe. There was a slight dry cough with soreness of the chest on coughing. Anorexia was marked; there was no nausea or vomiting. No other symptoms were described. The patient became concerned about the possibility of meningitis and sought examination on the morning of February 20th.

After physical examination disclosed a small lesion on the abdominal wall the patient recalled that ten days earlier, while at work in a pharmaceutical research laboratory, he had sustained what he thought was an insect bite. Itching was noted and, without inspecting the bite, he had scratched the wound occasionally during the afternoon. When he inspected the site in the evening a small red papule similar to a flea bite was present. This lesion thereafter was asymptomatic and was noted only while bathing. The patient was conducting experiments in a pharmacological laboratory where guinea pigs, dogs, rabbits, mice and rats were kept in the building; no unusual animal illnesses were noted and no strains of R. akari were being maintained anywhere in the plant. Mice were frequently observed in the building. No similar illness had been noted among the employees of the building but several associates had been ill during the winter with what was thought to be infectious mononucleosis.

The patient's past medical history was uneventful. He had resided in Indiana, Texas,

Kentucky, West Virginia and Illinois before coming to Pennsylvania. He had had mumps, measles, scarlet fever, chicken pox and pertussis in childhood, without sequellae. Appendectomy had been performed in 1943 and tonsillectomy in 1936.

talia, rectum and extremities revealed no abnormalities. There was moderate symmetric enlargement of axillary and inguinal lymph nodes. On the abdominal wall, in the right upper quadrant, was a sharply circumscribed red papule, 1.0 cm. in diameter, with a large



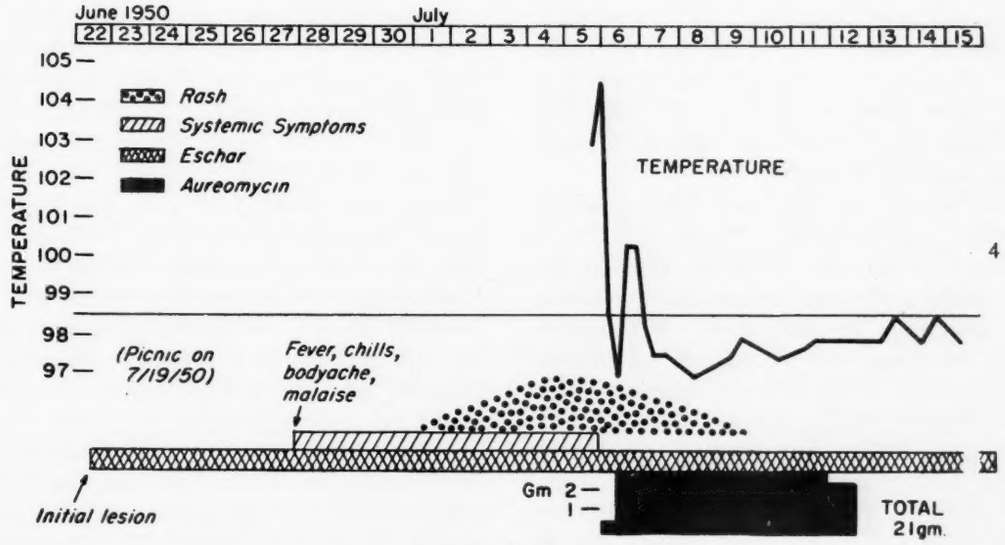


FIG. 3. Case III.
FIG. 4. Case IV.

Physical examination on admission to the hospital revealed a moderately uncomfortable, flushed but not acutely ill young man. The temperature was 100.2°F., pulse 92 and respirations 24. There was punctate erythema of the soft palate and the posterior third of the hard palate. There was moderate enlargement and tenderness of the cervical lymph nodes, most marked along the left sternocleidomastoid muscle. Examination of the chest was negative and the heart was normal. Blood pressure was 120/74. Examination of the abdomen, genicentral black eschar or punctum. No evidence of generalized eruption could be detected.

Initial laboratory studies included a normal hemoglobin and urinalysis. Leukopenia was present. Agglutination tests against typhoid, salmonella, brucella and proteus OX19 were negative. Blood culture showed no growth.

The crusted center of the bite lesion was removed and examined microscopically for insect residua; none was found. Serum from the superficially abraided papule was examined under the darkfield microscope, and stained for

micro-organisms and inclusion bodies. No unusual findings were noted.

The possibility of rickettsialpox was considered and therapy was withheld in order to observe the patient for development of a rash. Although not appearing seriously ill, the patient had severe shaking chills at 4 P.M. on February 20th and 4 A.M. on February 21st. Right axillary and inguinal lymph node enlargement and tenderness became more marked. No evidence of a rash could be detected, and at 6 P.M. aureomycin treatment was instituted, 250 mg. every six hours. At 9 P.M. a dozen fine erythematous macules were observed over the chest in front and in back. A chill of moderate severity occurred at 4 A.M. on February 22nd. The eruption was somewhat less prominent by morning and faded during the day so that examination at 2 P.M. revealed only suggestive faint macules. Lymph nodes were distinctly smaller and less tender, and the temperature rise that afternoon was not accompanied with chills. The patient thereafter was afebrile and asymptomatic except for fatigue on slight exertion. Aureomycin therapy was continued until February 28th, for a total of 7 gm. The initial lesion was present for one week after discharge from the hospital.

A blood count on February 24th revealed 4,200 white cells of which 42 per cent were lymphocytes, a majority of which showed the atypical nuclei and foamy basophilic cytoplasm seen in infectious mononucleosis. Heterophile antibody reactions were reported as follows: 1:14 on February 20th; 1:28 on February 24th; 1:56 on March 3rd; 1:14 on March 10th. Cephalin flocculation tests were 3+ on March 3rd, 10th and 29th, negative on July 15th. Thymol turbidity test on March 10th showed 1.3 units, with 3+ flocculation. Similar results were obtained on repetition on March 29th. A bromsulphthalein test showed no retention of dye and serum albumin and globulin were normal.

The patient was considered to have infectious mononucleosis until serologic studies repeated eighteen days after admission revealed a negative heterophile antibody test and strongly positive rickettsial complement fixation tests. (Table I.)

Comment: The appearance of constitutional symptoms five days after the initial lesion is compatible with rickettsialpox. The inguinal and axillary adenopathy, which was more marked on the right side where the abdominal lesion was located, presumably represented the regional adenitis of the lesion. The headache, stiffness and pain in the neck which suggested early meningitis was also noted in Case I. The secondary rash appeared eleven days after the initial lesion and six days after the constitutional symptoms began. The eruption was probably modified and prevented from assuming its characteristic appearance by aureomycin.

CASE IV. (Fig. 4.) J. S., a thirty year old white woman, was well until June 22, 1950, when she noted a lesion "resembling an infected hair follicle" on the anterior surface of the right ankle. She had walked barefooted in the grass on June 19, 1950, while on a picnic near Croydon, Pennsylvania, a suburban town on the Delaware River about four miles north of the Philadelphia city limits. She was not aware of any insect, animal bite or trauma to the ankle. The lesion on the ankle became necrotic, crusted and black. On June 28th she complained of fever, chills, slight headache, malaise and generalized aches and pains. She was treated with penicillin on June 28th. On June 30th she vomited. A rash appeared on the left upper arm on July 1, 1950, which gradually spread over all the extremities and sparsely over the body. Three days later, July 4th, the patient had severe headaches, chills and body aches. She was admitted to the Philadelphia Hospital for Contagious Diseases on July 5th with the diagnosis of Rocky Mountain spotted fever. The patient lives in the Richmond section of Philadelphia, four blocks from Cases I and II. (Figs. 5 and 6.)

Examination revealed a well developed and well nourished white woman, in no acute distress, with a temperature of 103°F., pulse 100 and a respiratory rate of 22. The significant findings were limited to a black eschar with an erythematous base over the anterior surface of the right ankle, and a discrete papular erythematous rash over the arms and legs with a few lesions scattered over the torso and face. Regional lymphadenitis was not apparent. The impression on admission was rickettsialpox or Rocky Mountain spotted fever.

An X-ray of the chest was normal. The red blood cell count was 3.71 million; white blood cell count 7,100 with 60 per cent neutrophiles, 38 per cent lymphocytes, 2 per cent eosinophiles and 11 gm. hemoglobin. Urinalyses were essentially normal. Agglutination tests for

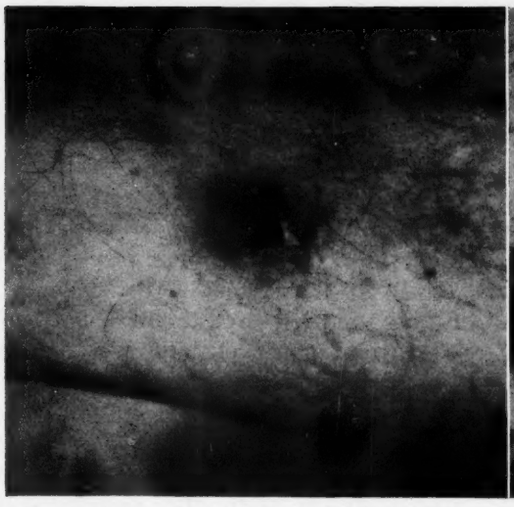


FIG. 5. Case IV. Initial lesion of Rickettsialpox.

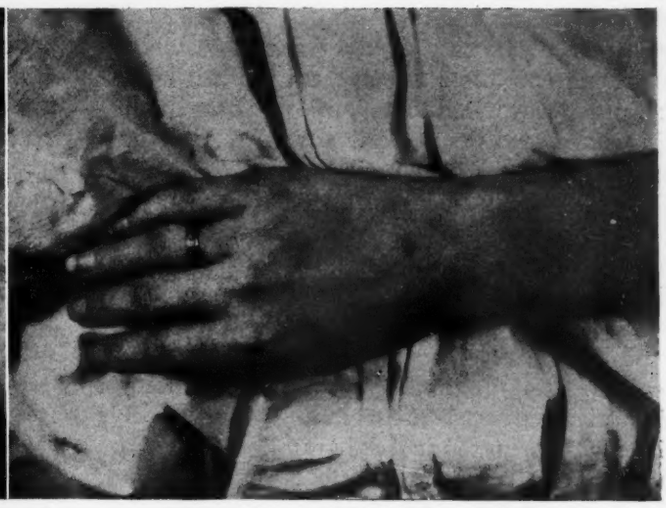


FIG. 6. Case IV. Rickettsialpox rash.

typhoid O, H, paratyphoid A, B, and Bacillus abortus were negative.

Aureomycin was started on July 5th 500 mg. every four hours until July 12th, the total dosage being 21 gm. over a seven-day period. The temperature dropped precipitously to normal within eight hours but did not remain normal until July 7th. On July 6th it was noted that the rash had faded and, although still weakened, the patient felt improved. The patient was free of complaints by July 10th, at which time the rash was completely faded and the base of the eschar on the ankle was erythematous. She was discharged on July 15th. On August 1st the eschar was still present but smaller in size.

Comment: The distribution of the rash, the possible occurrence of a tick bite on the picnic and the Weil-Felix reaction suggest Rocky Mountain spotted fever. On the other hand, the presence of a primary lesion and the fact that the patient resides in the vicinity of Cases I and II favor rickettsialpox. It is unlikely that this patient was infected with R. akari on June 19th on the picnic as the incubation period of rickettsialpox from mite bite to initial lesion is probably longer than three days.[1] The incubation period of rickettsialpox from the time of exposure to the appearance of the lesion in a laboratory worker was seven days, and twelve days to systemic symptoms.[11] According to Parker, the incubation period of Rocky Mountain spotted fever from the tick bite to systemic symptoms may be as short as three days but initial lesions rarely occur.[10] The tick bite lesion, when it occurs, does not resemble the lesion described in rickettsialpox. Ticks may leave puncture marks or a hemorrhagic area at the site of the bite in Rocky Mountain spotted fever. Occasionally, bacteria may be introduced followed by abscess formation.[10] Although initial lesions have been observed occasionally in Rocky Mountain spotted fever,[11] the characteristic eschar, the agglutinins for proteus OX19 in only a low titer favor the diagnosis of rickettsialpox or a related rickettsia. The patient lives within four city streets of Cases I and II, indicating the probability of an endemic focus of rickettsialpox in the community.

EPIDEMIOLOGIC SURVEY

When the mother of the first patient, Mrs. M. S. (Case II), was admitted to the Philadelphia Hospital for Contagious Diseases (on June 3rd) with a similar illness, the Division of Communicable Disease Control, with the cooperation of the Virus Diagnostic Laboratory, set up a program to survey the neighborhood of these patients and of another patient with Rocky Mountain spotted fever in Frankford, an adjacent community. Forty-nine homes were canvassed in the Frankford and Richmond sections of the City. In addition to the regular identifying data, information concerning the presence of scabs or rashes, travel habits and illnesses occurring in the preceding three years was obtained. Of 274 persons interviewed, blood specimens were obtained from eighty. Studies failed to reveal any cases of rickettsial infection not already known to the Health Department.

The Richmond area is one of the older sections of the City and is situated near the Delaware

River. The area is industrial in character with most of the homes owned by the occupants. Housing conditions are average, with very few substandard homes. The home of Cases I and II is located next door to a livery stable. Attempts to trap mice and mites in this stable and the home, however, were unsuccessful. Since the search for mice and mites could not be carried out at the time the patients became ill and the investigations were delayed for several months, the failure to demonstrate the presence of the animals and vectors does not rule out their existence in the area. Adequate epidemiologic investigation was, unfortunately, not possible in the pharmaceutical laboratory where Case III apparently became infected.

LABORATORY STUDIES

Materials and Methods

Rickettsial antigens: Antigens* of the "soluble" type were used. One series of tests with washed rickettsial antigens was performed for us through the kindness of Dr. J. E. Smadel at the Army Medical Department Research and Graduate School.

Proteus antigens: Proteus OX19 antigen* for rapid slide test, OX2, and OXK for the "test tube" agglutination tests were made available to us through the courtesy of the Bureau of Laboratories, Department of Health, Commonwealth of Pennsylvania.

Methods: The complement fixation test was performed by the method previously described.[12] Agglutination tests were made by several methods:

(1) Rapid slide agglutination: This was carried out according to the technic recommended by directions accompanying the antigen.

(2) The "shake and centrifuge" method: In this procedure serial dilutions of serum in 0.5 ml. amounts were mixed with 0.5 ml. amounts of antigen in recommended dilution. The tubes were first shaken in the Kahn shaker for three minutes and then centrifuged at approximately 2,000 RPM for twenty minutes. The degree of agglutination was determined by observing the manner in which the sedimented bacteria became resuspended on gently tapping the tube.

(3) Slow agglutination at 37°C.: The usual procedure of mixing serial serum dilutions in 0.5 ml. amounts with a single dilution of 0.5 ml. amounts of antigen. Tubes were incubated at 37°C. for two and one-half hours and then placed in the refrigerator for approximately sixteen hours. The tubes were again incubated at 37°C. for fifteen minutes before reading.

(4) Slow agglutination at 52°C.: Same as method 3 except that the tubes were kept at 52°C. for the entire period of incubation (approximately nineteen hours). The results of the various serologic tests are shown in Table I.

All of the patients showed a rise in antibody titer to the agent of rickettsialpox as demonstrated by the complement fixation test. When tests were made with the antigen of Rocky Mountain spotted fever a reaction was also noted. Usually this reaction was of about the same magnitude as with the rickettsialpox antigen. Occasionally, especially in one of a series of tests on serum No. 2 of Case I, R. S., a pronounced difference in titer was noted in favor of the rickettsialpox antigen (1:64 vs. 1:8). It is quite obvious that the complement fixation test with these two antigens does not differentiate between Rocky Mountain spotted fever and rickettsialpox. A similar serologic pattern was obtained on specimens of Case I, R. S., and Case II, M. S., by Dr. Morris Greenberg of the Department of Health, New York City. None of the sera tested reacted with the antigens of epidemic and murine typhus. As shown in Table I, the complement fixation test with washed antigens, as performed at the Army Medical Department Research and Graduate School, yielded stronger reactions with the rickettsialpox than with the Rocky Mountain spotted fever antigen.

The agglutination test with both proteus OX19 and OX2 showed reactions only in low dilutions. These sera, as well as several from patients with Rocky Mountain spotted fever, gave negative results with proteus OXK.

Table II shows comparative agglutinations in the four patients described in this article with four patients with a diagnosis of Rocky Mountain spotted fever selected at random and tested at the Virus Diagnostic Laboratory. It is unfortunate that more specimens could not be tested and that many of the available sera were in quantities too small to permit additional tests. The highest titers were shown by the "shake and centrifuge" method; the 52°C. incubation gave higher titers than the 37°C. incubation method. The sera shown in Table II were obtained about two to three weeks after

* A product of Lederle Laboratories, New York, N. Y.

the onset of illness. It appears that the Weil-Felix reaction is helpful in the differential diagnosis—two of the patients with Rocky Mountain spotted fever showed relatively high antibody titers—but cannot be relied on as a critical differential procedure. More extensive work on the serologic pattern as it pertains to the antirickettsial and antiproteus antibodies in these rickettsial disease is clearly indicated.

TABLE II
COMPARISON OF THE WEIL-FELIX REACTIONS OF OUR PATIENTS WITH FOUR CASES OF ROCKY MOUNTAIN SPOTTED FEVER

Rocky Mountain Spotted Fever			Rickettsialpox		
Maximum Weil-Felix Titers			Maximum Weil-Felix Titers		
No.	0X19	0X2	Patient	OX19	OX2
1,827	40	20	Case I, R. S.	40	80
1,621	40	80	Case II, M. S.	40	40
3,865	320	160	Case III, C. A. R.	20	20
1,841	640	40	Case IV, J. S.	80	20
Geometric mean	135	56	.....	40	33

Figures are the reciprocal of the dilutions.

OBSERVATIONS

Since the reservoir of rickettsialpox, Mus musculus, and the vector, Allodermanyssus sanguineus, are so widespread, the disease in man is probably more prevalent than heretofore recognized.[9] With increased awareness of the existence of the disease by physicians, and the knowledge that the disease is not confined to New York City, more cases presumably will be diagnosed. Typically, the first manifestation of rickettsialpox is a primary lesion at the portal of entry of the causative agent. Constitutional symptoms usually appear within one week and are followed in several days by an eruption. Complement fixing antibodies for the Rocky Mountain spotted fever-rickettsialpox group appear during convalescence while agglutinins for Proteus vulgaris (Weil-Felix) do not as a rule appear in significant titers.

The primary lesion was absent in one of twelve cases observed by Barker,[7] and in four of eighty-six cases studied by Greenberg et al.[3] Rose was unable to find a primary lesion in six of thirty-five patients reported by him.[11] A primary lesion in the presence of a generalized maculopapular eruption is good evidence for the presumptive diagnosis of rickettsialpox. The primary lesion may be obscurely located in the scalp, nostrils or on the genitalia. One must keep in mind the possibility of the disease occurring without a primary lesion, the portal of entry being other than the skin, such as perhaps the respiratory system. A primary lesion, somewhat resembling the eschar of healing cutaneous anthrax, was seen in three of our patients.

The similarity of this disease to varicella has probably been overemphasized. None of our patients had vesiculation of the rash to suggest the diagnosis of varicella. Treatment with specific antibiotics apparently influences the rash and may abort or modify the rash if used early in the disease. Confirmation of the diagnosis is obtained by isolation of the R. akari or by demonstrating a rising antibody titer in the patient's serum for rickettsialpox antigen. Isolation of R. akari from the patient's blood has been achieved occasionally but is not practical as a routine diagnostic procedure. The major difficulty with the serologic test is that cross reactions occur between rickettsialpox and Rocky Mountain spotted fever antigens.[11] Serologic findings should not be considered significant unless the complement fixation test for rickettsialpox is positive in a high titer and agglutination for proteus OX19 (Weil-Felix test) does not occur in higher than 1:80 dilution.

Apparently, heterophile antibodies may appear temporarily in low titers in rickettsialpox, as noted by Barker[7] and as observed in three of our patients. Other features characteristic of infectious mononucleosis were observed in one patient (Case III): the generalized lymphadenopathy, atypical lymphocytes and a positive cephalin flocculation reaction. It would appear desirable to perform serologic tests for rickettsial infection in patients who appear to have infectious mononucleosis, especially in those patients who exhibit a marked response to treatment with aureomycin, terramycin or chloromycetin.

One of our patients (Case IV) presents clinical features and borderline laboratory findings of Rocky Mountain spotted fever and rickettsialpox. It is appreciated that Rocky Mountain spotted fever should be given serious consideration but the presence and duration of the

primary lesion and the residence of the patient strongly favor rickettsialpox. The distribution of the rash, which was heavy over the extremities and sparse over the torso, and the Weil-Felix titer (1:80), which was the highest of the four patients, suggest Rocky Mountain spotted fever.

Absolute proof of the etiology of rickettsialpox has not been established in any of our patients. Whereas in an endemic area a clinical and serologic diagnosis may suffice, in an area where this disease has never been reported, proof of its existence actually requires the demonstration of R. akari in the human host, the vector or the reservoir. It was impossible to do this in our studies. However, the following features and findings strongly suggest that these patients had rickettsialpox: (1) three of the four patients from the same city area; (2) two cases in one household; (3) no history of tick bites; (4) typical eschar in three of the four patients; (5) negative Weil-Felix tests in dilution higher than 1:80; (6) positive complement fixation tests with antigens of the Rocky Mountain spotted fever-rickettsialpox group in high dilutions; (7) relatively strong complement fixation reaction with washed rickettsialpox antigen.

SUMMARY AND CONCLUSIONS

1. Four patients with clinical and laboratory findings compatible with rickettsialpox, apparently incurred in metropolitan Philadelphia, are reported.

2. Clinical and serologic evidence for the diagnosis included eschars in three patients, rashes and constitutional symptoms, significant titers against washed and unwashed rickettsialpox antigen, and slight or negative Weil-Felix reactions.

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Octamethyl Pyrophosphoramide in the Therapy of Myasthenia Gravis*

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THE value of alkyl phosphates, notably tetraethylpyrophosphate, is well established in the treatment of myasthenia gravis. While these compounds were used first as insecticides, their powerful cholinergic action in mammals suggested that they might be of value in the therapy of this condition. Although TEPP has proved to be the most effective and practical anticholinesterase drug to date, certain of its properties make it desirable to find a still more reliable therapeutic agent. The undesirable qualities of TEPP have been enumerated by Stone and Rider[1] and include (1) narrow range between toxic and therapeutic dose; (2) serious toxic manifestations of central nervous system origin, including convulsions and unconsciousness; (3) marked instability of the compound with prompt hydrolysis to inert diethyl acid phosphate on contact with water.

Similar objections were raised by the same authors to neostigmine and included brief duration of action (two hours), undesirable side effects, erratic control of severe cases and occasional necessity for parenteral administration.

Octamethyl pyrophosphoromide (OMPA) is a colorless, viscous liquid when approximately 99 per cent pure. Further purification results in solidification. The compound is miscible with water in all proportions and is soluble in most organic solvents. It is not hydrolized by water or alkali and is therefore relatively stable. (Fig. 1.)

In their search for a cholinergic organic phosphate with a predominantly peripheral site of action, DuBois et al.[2] found that octamethyl pyrophosphoramide, although comparatively ineffective *in vitro*, is a powerful anticholinesterase agent *in vivo*. This is due to the fact that it is converted by the liver under aerobic conditions into an anticholinesterase substance. This substance has, as yet, not been identified, although DuBois believes that the reaction probably involves oxidation. In contrast to the action of alkyl phosphates (TEPP), brain cholinesterase is not affected *in vivo*, apparently due to inability of the active metabolite to gain access to the central nervous system. The inhibition of peripheral cholinesterase appears to be irreversible *in vitro*.

Symptoms of acute poisoning in dogs after a lethal dose are principally signs of extreme gastroenteric stimulation followed by muscular fibrillation, tremors, weakness and, finally, prostration, bradycardia and respiratory depression. In contrast to TEPP there are no convulsions, depression of consciousness or reflex changes. Atropine will prevent or abolish all peripheral manifestations of parasympathetic stimulation and will substantially increase the LD_{50}.

These properties make it evident that OMPA is an effective cholinergic drug without the undesirable properties of TEPP and neostigmine. Its effects in patients with myasthenia gravis have been reported by Rider, Schulman, Richter, Moeller and DuBois.[3]

At the present time, sixteen patients with myasthenia gravis have received OMPA at the John Sealy Hospital. From Table 1 it will be seen that the duration and severity of their disease varied considerably as judged from the prostigmine requirement. Cases 1, 3, 8, 9, 10 and 13 were almost incapacitated and were frequently unable to care for themselves. Case 3 had been confined to bed three years and was unable to walk, even with assistance. Case 9 was frequently unable to take her own neostigmine, orally or parenterally. The conditions of the remaining ten patients were moderately severe to mild.

Duration of illness ranged from seven months to eleven years and averaged four years. The severity of the disease was not positively corre-

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lated with its duration, nor was the response to OMPA. Significant spontaneous remission had occurred in only one instance (Case 12). Temporary improvement occurred coincidentally with previous therapeutic efforts in some cases.

Five patients (31 per cent) had had thy-

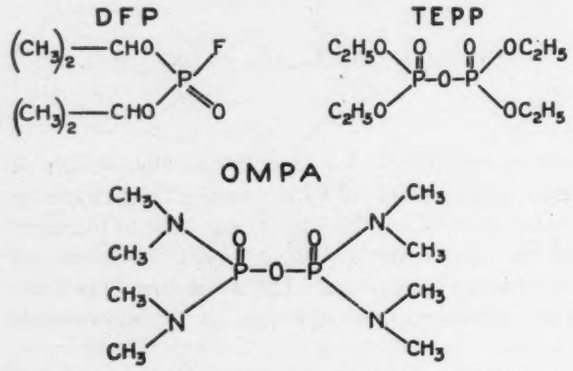

FIG. 1. Chemical structures of three long-acting anticholinesterase agents.

mectomies. Two operations were performed at John Sealy Hospital and three elsewhere. None of the glands showed malignant changes. Temporary improvement occurred postoperatively in three cases and has persisted to lesser degree in one patient (Case 4).

Four patients who have received OMPA have died; in one instance TEPP had been substituted at the time of death. These will be discussed individually.

Of sixteen patients taking OMPA, all have found it to be the most satisfactory preparation yet obtained. The most significant improvement over previous drugs is its uniformity of action with maintenance of strength at corresponding levels throughout the day. Patients are able to go about their morning toilet and to some extent their regular daily occupations. When taking only neostigmine some of them frequently were unable to take their first dose on awakening because of weakness or difficulty in swallowing. This serious problem has not arisen after adequate dosage of OMPA has been attained.

The amount of improvement obtained is variable. Our patients are told that they can expect to attain no more than their maximum strength and endurance provided by neostigmine, but that the improvement will be maintained throughout the day. In thirteen cases the patients have felt subjectively stronger while taking OMPA than their maximum strength on neostigmine. This, they believe, is independent of the more uniform action. The difference is less marked when the results using TEPP are compared. Of seven patients who have received both drugs, four felt stronger when using OMPA and two when taking TEPP. One (Case 2) was unable to tolerate an effective dosage of TEPP. One patient (Case 4) who preferred TEPP was forced to use OMPA because of toxic manifestations (convulsions) of the former drug.

The second important advantage of OMPA, primarily over TEPP, is inherent in its pharmacologic property of being exclusively peripheral in action. It is this factor that accounts for the lack of serious toxic symptoms. In two of our patients (Cases 4 and 7) generalized convulsions occurred while receiving TEPP. The added symptoms of weakness may, at times, create confusion as to whether the remedy or the disease is responsible. On the other hand, the first toxic effect of OMPA has uniformly consisted of abdominal cramping and diarrhea. Further increases in dosage lead to increase of these symptoms rather than to more serious toxic manifestations, without further enhancement of muscle strength. Two other side effects of OMPA have been reported. Moderate sweating occasionally occurs and has been noticeable in four patients. Two patients have experienced difficulty with distant vision apparently caused by spasm of the ciliary muscles (Cases 12 and 15). This has not occurred after slight reductions of dosage.

Another advantage of OMPA over TEPP lies in its chemical stability. Two of our patients found it impossible to maintain effective control without toxic effects using TEPP because of variability in the potency of that drug. Two who traveled were unable to keep their supply of TEPP refrigerated. These faults are not inherent in OMPA which is relatively stable.

OMPA* is dispensed as a 1 per cent solution in distilled water or propylene glycol, the latter being more satisfactory. It requires no refrigeration or other special precautions in handling to prevent deterioration. Patients are taught to measure it accurately in a dry tuberculin syringe and to dilute it in a few ounces of water or fruit juice. No neostigmine is permitted within three hours of the administration of OMPA since there appears to be antagonistic action within this period. It is probable that

* OMPA has been made available to us in 25 mg. ampules by Eli Lilly and Co.

neostigmine given within one and one-half or two hours of the administration of OMPA inhibits the effective action of the latter by combining with cholinesterase. Since this combination is unstable, the cholinesterase is released after OMPA metabolite has been destroyed or excreted and full effects are not obtained.

strength, four 15 mg. tablets being the maximum requirement (Case 3). Patients receiving OMPA find that the side effects of neostigmine are greatly potentiated, the most troublesome being abdominal cramps and sweating. Caution therefore should be observed in the parenteral administration of neostigmine to patients

TABLE I

EFFECTS OF OCTA-METHYL-PYROPHOSPHORAMIDE ON MYASTHENIA GRAVIS

Case	Patient	Sex and Age	Weight	Duration of Disease (yr.)	Thymectomy	Prostigmine Dose mg./day		Mg. OMPA Dose /day	Duration of Therapy (mo.)	Activities
						Before OMPA	After OMPA			
1	P M B	F, 22	157	3	Yes	300	0	32.5	19	Very slightly restricted
2	B S	F, 35	122	10	No	450	0	23	17	Slightly restricted
3	B W W	M, 22	180	6	Yes	810	60	36	18	Slightly restricted
4	E L P	F, 28	119	2	Yes	120	0	14	20	Unrestricted
5	W A R	M, 50	155	11	No	450	30	28*	2	Died
6	A B	M, 23	134	8 mo.	No	150	..	22*	..	Died
7	L F R	M, 55	129	3	Yes	180	30	24	2½	Died
8	L P C	M, 41	180	7 mo.	No	300	0	24	10	Moderately restricted
9	B C	F, 23	122	5	Yes	410	15	20	3	Died
10	T H	F, 37	149	1	No	75	0	20	9	Moderately restricted
11	V J	F, 51	123	1	No	60	0	18	8	Unrestricted
12	W D	M, 36	146	6	No	180	30	22	18	Very slightly restricted
13	B J W	F, 24	115	3	No	180	0	14	7	Unrestricted
14	V H	F, 17	101	1	No	60	0	17	9	Unrestricted
15	H Y	M, 55	130	2	No	180	0	21	8	Moderately restricted
16	L B	M, 54	155	4	No	...	15	22	12	Slightly restricted

* Left hospital before full dosage attained

The dose in our cases ranged from 14 to 36 mg. per day, with an average of 24. This amount is given in divided doses after the morning and evening meals. In initiating therapy patients are begun on 5 mg. twice a day. The dose is increased by 2 mg. daily until 18 mg. per day are given. After two or three days the dose is again increased by 1 mg. every two or three days until abdominal cramps or diarrhea occur. These symptoms have appeared in all patients receiving a therapeutic dose and are used as an indication of full therapeutic effect. In our experience, these symptoms are of more value in regulating dosage than are serum and red cell cholinesterase levels. The untoward symptoms are usually well controlled by adequate doses of belladonna. In only one instance (Case 4) has it been necessary to reduce the dose from maximal response because of diarrhea. Some of the patients require the use of added doses of neostigmine for maximum

receiving OMPA. The following cases are illustrative:

P. M., a colored female age twenty-two, had myasthenia gravis of three years' duration. She experienced a stormy course after thymectomy, as reported in detail elsewhere. Before TEPP became available she required 200 to 300+ mg. of neostigmine daily and her activities were severely limited. On TEPP she was able to be "up and around almost at will" but could walk only short distances. She required 60 mg. of neostigmine bromide daily, in addition to TEPP. After changing to OMPA she experienced additional increase in strength, and was able to walk several blocks without undue fatigue. She performed all her housework and has undergone two uneventful pregnancies. She no longer requires additional neostigmine. She feels somewhat weaker at the time of her menstrual periods and requires an extra 5 mg. of OMPA for several days. Her only side effects

are abdominal cramps which are controlled by atropine. This case represents excellent response to anticholinesterase drugs. Although not entirely normal, she is able to perform her household chores and care for her increasing family.

B. W., a white male age twenty-two, had myasthenia gravis of six years' duration. He had a thymectomy in 1947 with transient improvement. His dose of oral neostigmine ranged as high as 720 mg. plus 3 mg. parenterally. This dose was minimal because of financial conconsiderations and he was bedridden. He experienced rapid fatigue on talking and writing. He has obtained continued improvement on OMPA and is able to drive a jeep 80 miles a day over country roads and is self-supporting. His OMPA ration is supplemented by 45 to 60 mg. of neostigmine bromide daily.

Both these cases represent outstanding results. While improvement has been immediate and remarkable, the possibility of spontaneous remission must be borne in mind since myasthenia gravis characteristically runs a variable course.

E. P., a white female age twenty-eight, had myasthenia gravis of two years' duration. She required 120 mg. of oral neostigmine per day before thymectomy. Partial remission occurred after operation, with increased strength from 90 mg. of neostigmine. She experienced excellent response to TEPP plus 75 mg. of neostigmine and felt entirely normal. It was necessary to change to OMPA because of convulsions and coma. She was able to take only 18 mg. of OMPA per day, and later required reduction to 14 mg. for relief of severe cramping and diarrhea. On this dose she feels less strong than with TEPP but requires only occasional neostigmine. She is able to perform all her housework except ironing, and is much stronger than when using neostigmine alone. This is the only patient who required reduction of optimal dose due to untoward effects. Her disease was not severe.

Four patients in our series have died, only one while in John Sealy Hospital.

L. F. R., a white male age fifty-five, had myasthenia gravis of three years' duration. Before thymectomy he required 180 mg. of neostigmine bromide daily. After thymectomy he was given TEPP and required only 30 mg. of neostigmine a day. He was able to resume his occupation as a barber. He developed homologous serum hepatitis after surgery and did not recover normal liver function, nor was he able to return to work. He was changed to OMPA and felt somewhat improved for a short period but continued to lose weight and strength. He was rehospitalized two months before his death and was found to have severe liver dysfunction, although not jaundiced. His strength improved considerably on vigorous lipotrophic and supportive care but his liver function studies revealed increasing liver involvement. He was discharged from the hospital only to return after one week, having collapsed at home where he had contracted influenza. On the first hospital night he experienced respiratory failure but responded to the intravenous administration of 1 mg. of neostigmine. Because of the possibility of incomplete conversion of OMPA to the active metabolite by his damaged liver, OMPA was discontinued and TEPP begun. This medication was poorly tolerated, and he complained continually of nausea, abdominal cramps and drenching sweats. Efforts to control his respiratory infection were disappointing. After he appeared to be improving spontaneously he suddenly became weak, experienced marked respiratory distress which failed to respond to parenteral neostigmine, and rapidly expired.

At autopsy no evidence of recurrence of the thymic tumor was noted. The liver weighed 1,400 gm. Gross appearance was indistinguishable from portal cirrhosis. Microscopic examination, however, showed typical findings of chronic active hepatitis.

This case was one of severe myasthenia complicated by equally severe progressive liver disease. To this was added epidemic influenza. This type of death, rapid respiratory failure, is characteristic of myasthenic crisis.

B. C., a white female age twenty-three, had myasthenia six years. Transient partial remission occurred after thymectomy. At the time of admission she had required 30 mg. of neostigmine every two hours, day and night, and was confined to her home. She had become progressively weaker during the five months before admission, at times being unable to swallow her first morning dose of neostigmine and to give herself an injection. On OMPA she experienced approximately 50 per cent increase in strength which was maintained through the twenty-four hours. Two months after discharge from John Sealy Hospital she expired in a myasthenic crisis.

Two other deaths occurred elsewhere in patients who had left the hospital before the dose of OMPA had been increased to tolerance.

Description of their deaths by attending physicians paralleled the one described previously and consisted of sudden respiratory weakness unresponsive to parenteral neostigmine.

It is necessary to conclude from these fatal cases that long-acting anticholinesterase drugs will not protect against crisis of myasthenia. At such times, neostigmine likewise has failed to elicit response.

SUMMARY AND CONCLUSIONS

Experiences with a new drug, OMPA, in the therapy of patients with myasthenia gravis are described. OMPA possesses most of the desirable characteristics and none of the important undesirable properties of TEPP and neostigmine. These include oral administration, prolonged and uniform action, chemical stability, absence of central nervous system toxic effects and ease of determining maximum dosage. Fifteen of sixteen patients had less limitation of activity following OMPA than with the use of any other drug. It is the most satisfactory preparation we have found in managing patients with myasthenia gravis.

Fatalities which occurred indicate that wholly effective treatment for myasthenia gravis has yet to be found.

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Triethylene Melamine in Clinical Cancer Chemotherapy*

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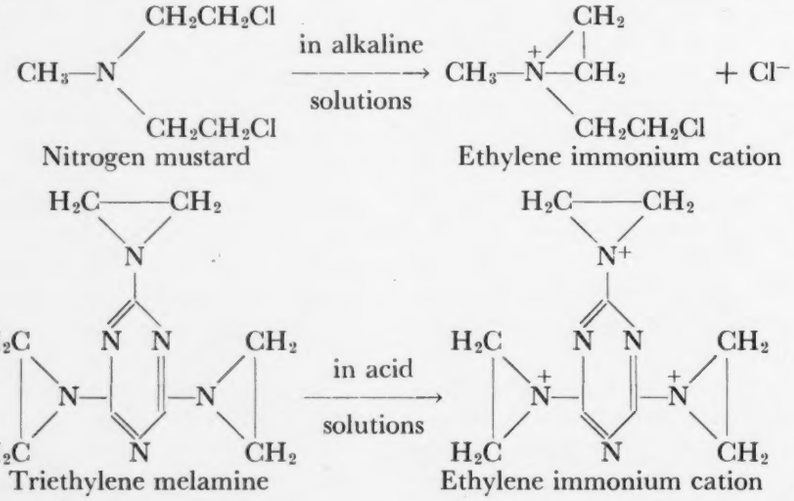
FOLLOWING the introduction of nitrogen mustard (HN_2) as a chemotherapeutic agent for the management of disseminated malignant lymphomas and inoperable bronchogenic carcinoma in 1943, hundreds of closely related compounds have been synthesized and studied experimentally. Although a small number of these demonstrated certain advantages over HN_2 in the laboratory, clinical evaluation failed to justify their substitution for the commonly employed nitrogen mustard. Recently, however, a related chemical compound has been examined both in the laboratory and in clinical therapy which offers significant advantages over HN_2 in selected cases. This drug, triethylene melamine, is effective after oral administration and the incidence of immediate toxic manifestations is appreciably less than with the intravenous nitrogen mustard.

The present report† will summarize the experience of this clinic in forty-four cases treated during the past two years. At the outset it can be stated that the drug does not cure any malignant disease. Emphasis will be placed on the indications, limitations, dosage and toxicity. The experimental observations which provided the basis for clinical trial have been reported by Rose[1] and Philips,[2] and detailed discussion of the drug in cancer chemotherapy has been presented by Karnofsky et al.[3] and Wright et al.[4]

METHOD OF ADMINISTRATION AND DOSAGE

Triethylene melamine has routinely been administered orally together with 2 gm. of sodium bicarbonate. The drug and the alkalinizing agent are given on awakening in the morning and breakfast is delayed for two hours thereafter. In our experience sodium bicarbonate appears to be an important adjuvant. The



rationale for its use rests on the chemical characteristics of the drug when in solution. This can most readily be understood from the above

† The triethylene melamine used in this investigation was generously supplied by Dr. J. M. Ruegsegger, Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

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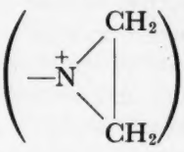
reactions which contrast nitrogen mustard and triethylene melamine.

It will be recalled that the biologic activity of nitrogen mustard depends upon an intramolecular cyclization with the formation of an ethylene immonium cation  . This transformation occurs with great rapidity in alkaline solutions and only very slowly in acid solutions. Triethylene melamine has three ethylene imine radicles but the formation of the biologically active ethylene immonium cation takes place readily only in acid solutions.

In order to prevent the formation of this chemically reactive form of triethylene melamine in the acid gastric juice, sodium bicarbonate is given. This theoretically prevents reaction of the drug with substances in the gut and allows systemic absorption of the entire dose. That these theoretic chemical considerations have practical clinical application is indicated by the fact that we have found the dosage to be quite constant from patient to patient. In the reported series of Karnofsky and of Wright the dosage varied from 10 to over 100 mg. Our method of administration of triethylene melamine differs from that of these investigators only in the addition of sodium bicarbonate. The variability in dosage in other clinics may be reasonably explained by partial and unpredictable reaction of a proportion of the administered dose in the gastrointestinal tract. Since it is necessary to wait ten days to two weeks to determine the extent of hematopoietic depression, it is obvious that titration of dosage against therapeutic effect and toxicity may be difficult and prolonged; and therefore any measure which reduces dose variation is useful.

In our experience the addition of sodium bicarbonate to the regimen has made it possible to restrict the dosage range to from 5 to 15 mg. In patients with Hodgkin's disease without bone marrow depression, it is our practice to give 5 mg. of triethylene melamine with 2 gm. of sodium bicarbonate on two successive mornings. In the majority of cases this constitutes the therapeutic course. In an occasional patient an additional 5 mg. of TEM may be given two weeks later if full therapeutic effect has not been achieved and leukopenia has not developed. We have confirmed the observations of Karnofsky that lymphosarcomas and chronic lymphocytic leukemia are particularly sensitive to the drug. For these cases it is customary to give 2.5 mg. with bicarbonate on two successive days.

The single death due to triethylene melamine in our series was that of a young woman with fulminating Hodgkin's disease who received one injection of intravenous nitrogen mustard at a dosage of 0.2 mg. per kg. and then one week later was started on triethylene melamine. She received 20 mg. of this drug in a period of one week, with 2 gm. of sodium bicarbonate given with each 5 mg. of triethylene melamine. The temperature curve, which had been spiking daily to 105°F., returned to normal in one week and subjectively the patient was greatly improved. After a remission of two weeks, however, severe pancytopenia developed and she died with signs and symptoms of intracranial bleeding. This experience demonstrates the potency of the drug and indicates the narrow margin between therapeutic and lethal doses.

RESULTS

On the basis of the laboratory evidence which has demonstrated the close parallel in pharmacodynamic action of nitrogen mustard and triethylene melamine it was not considered necessary to explore extensively the therapeutic action of the latter drug against a broad spectrum of human tumors. The assumption that triethylene melamine would be most useful in the management of the malignant lymphomas and the chronic leukemias was borne out in our experience, and the reported observations of Karnofsky and of Wright lend further support to this conclusion.

Table 1 summarizes the forty-four cases studied together with a qualitative indication of the response to therapy. Improvement in patients with Hodgkin's disease was measured by decrease in peripheral and mediastinal lymphadenopathy, diminution of hepatosplenomegaly, drop of the sedimentation rate and fever, and subjective improvement. It was noted that the onset of remission following triethylene melamine was appreciably slower than following intravenous nitrogen mustard, usually occurring between ten and fifteen days after the completion of treatment. This delay in apparent therapeutic effect proved to be a disadvantage in the management of patients with high temperature and severe constitutional symptoms.

As was mentioned in the preceding section, the lymphosarcomas and chronic lymphocytic leukemias appear to be particularly sensitive to the cytotoxic action of triethylene melamine. In patients with these diseases there was a decrease in lymphadenopathy and peripheral

TABLE I
DISTRIBUTION OF CASES AND RESULTS OF TRIETHYLENE MELAMINE TREATMENT

Disease	No. of Cases	No. of Courses of TEM	No. Improved	Duration of Improvement Following a Course of TEM (Mo.)		
				1–3	3–6	6–12
Hodgkin's disease	24	36	20	23	5	4
Lymphosarcoma	7	11	6	6	2	2
Chronic leukemias	5	5	3	1	2	..
Bronchogenic carcinoma	4	6	3	3	2	..
Anaplastic carcinoma	1	1	0	..	..	..
Reticulum cell sarcoma	1	1	0	..	..	..
Ewing's tumor	1	1	0	..	..	..
Rhabdomyosarcoma	1	1	0	..	..	..

lymphocytosis following small doses of the drug. Improvement in these patients was subjective as well as objective, but impressive spontaneous rises of hemoglobin in the chronic leukemia patients was not observed.

The patients with bronchogenic carcinoma had decrease in cough, sputum, hemoptysis and chest pain. Objective changes measured by serial roentgenograms was not striking.

The toxic manifestations of triethylene melamine are qualitatively similar to nitrogen mustard but there are certain notable quantitative differences. Nausea occurred in twenty-two of the forty-four patients who received the drug and of these only thirteen had vomiting. This incidence and severity of gastrointestinal symptoms are to be contrasted with those of intravenous nitrogen mustard, which produces nausea and vomiting in virtually every patient. When gastrointestinal toxicity does occur after triethylene melamine, it usually appears seven to ten hours after the drug has been given. Leukopenia of 2,000 or less was noted six times; thrombocytopenia of 50,000 or less occurred in five patients and purpura was present in three of these cases. Maximum depression of the formed elements of the blood was observed fourteen days after administration of triethylene melamine. There was one death in this series directly ascribable to triethylene melamine and this patient manifested pancytopenia with intracranial bleeding.

COMMENTS

It has been pointed out that triethylene melamine is effective against the same groups of neoplastic disease in which nitrogen mustard is active. In spite of the fundamental similarities in action there are, nevertheless, certain subtle differences which permit a reasonable choice to be made between the two drugs in individual patients. Thus in Hodgkin's disease we have found that triethylene melamine is particularly useful in the management of the patient with disseminated disease who is not acutely ill due to severe constitutional symptoms. In this group of patients the drug may be given on an ambulatory basis with the expectation of minimal immediate toxicity and the anticipation of a therapeutic effect in from seven to fourteen days. In the patient with Hodgkin's disease with high temperature, severe malaise and weakness the onset of action of triethylene melamine is unduly delayed. These patients are more satisfactorily treated with intravenous nitrogen mustard which usually produces obvious improvement within forty-eight to seventy-two hours after completion of the course. In patients with giant follicular or lymphocytic cell lymphosarcoma triethylene melamine appears to us to be more effective than intravenous nitrogen mustard and the same holds true for chronic lymphocytic leukemia. These patients usually are not as desperately ill with systemic symptoms as some individuals with Hodgkin's disease until their disease has entered the final phase; the conditions, therefore, are optimal for ambulatory treatment with triethylene melamine. As has been mentioned, the dosage of the drug required is usually significantly less than for Hodgkin's disease or bronchogenic carcinoma.

Although we have stressed the ease of administration and the relatively minor severity of nausea and vomiting, it must be recognized that triethylene melamine is an extremely toxic drug which can and does depress bone marrow function. If the chemical compound is given with sodium bicarbonate as has been described in this report, the dosage of 10 mg. should not be exceeded unless there has been no significant depression of the formed elements of the blood during ten days after the administration of the drug. If the blood counts are stable or only minimally depressed at this time, an additional

5 mg. may be given safely and once again the toxic and therapeutic effects observed for a ten-day period. Following a course of triethylene melamine in which there has been leukocyte depression, an interval of four to six weeks should elapse before additional chemotherapy with this drug or nitrogen mustard, or before instituting radiotherapy which might necessarily include the major hematopoietic areas such as the ribs, sternum or thoracolumbar spine.

SUMMARY AND CONCLUSIONS

The use of triethylene melamine, a drug with nitrogen mustard-like action, in the therapeutic management of forty-four cases of disseminated malignant disease is summarized. Although triethylene melamine produces toxic and therapeutic effects which are qualitatively similar to those of nitrogen mustard, it possesses the advantage of being active after oral administration and of causing significantly less nausea and vomiting. These properties facilitate treatment of ambulatory patients.

The rationale for administration of sodium bicarbonate simultaneously with triethylene melamine is presented, together with evidence which indicates that this regimen sharply decreases the variability of effective dosage from one case to another. Triethylene melamine has been employed to advantage in the treatment of patients with malignant lymphomas, chronic leukemias and bronchogenic carcinoma. The indications for using this drug or nitrogen mustard are discussed.

The toxicity of triethylene melamine in regard to bone marrow function is stressed. One fatality due to overdosage of the chemical compound with resulting pancytopenia is reported.

In appropriately selected cases and with full recognition of the potential toxic hazards triethylene melamine may be employed as an additional useful cancer chemotherapeutic agent.

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Review

Alcaptonuria and Ochronosis*

With a Report of Three Patients and Metabolic Studies in Two

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ALCAPTONURIA is a rare disorder of the metabolism of the amino acids, tyrosine and phenylalanine, characterized by the excretion of homogentisic acid in the urine. Approximately 200 cases have been described. It occurs most commonly in males and is believed to be transmitted as a recessive trait at birth.[1]

Boedeker[2] in 1858 was the first to recognize in urine a reducing substance with an affinity for oxygen in an alkaline medium which he called "alkapton." The term alcaptonuria was derived from this reaction. It was not until 1891 that this substance was isolated from the urine of an alcaptonuric[3] and identified as homogentisic acid. (Fig. 1.) Homogentisic acid is the only abnormal substance which these individuals excrete in the urine and it imparts certain chemical properties to it. The color of the freshly voided urine is not unusual if it is acid in reaction; if alkaline, it appears as some variant of brown or even black. When a specimen stands exposed to air, it gradually darkens at its upper surface and in time becomes black throughout. Of interest, particularly when the appearance of the urine is normal, are the reactions with Benedict's solution and ferric chloride. Homogentisic acid reduces Benedict's solution giving rise to a yellow or orange precipitate and a brown or black supernatant fluid. Failure to differentiate this reaction from that due to glucose may suggest the erroneous diagnosis of diabetes mellitus. This reaction can be differentiated from that due to glucose by the failure of homogentisic acid to ferment yeast and to rotate a beam of polarized light. When ferric chloride is added dropwise to the urine, an evanescent blue color appears. When strong alkali is added and the specimen is shaken, it promptly turns dark. All of the above reactions are presumptive evidence of alcaptonuria. Isolation of homogentisic acid from the urine is the only certain method of establishing the diagnosis.

Alcaptonuria is compatible with long life. Except for discoloration of the urine, there are no clinical manifestations until the second or third decade when ochronosis begins to appear. The term ochronosis was first used by Virchow[4] to describe the ochre-like color of pigment deposits in sections of tissues of a subject whom he examined postmortem. No clinical record of the patient was available. In its fully developed form, which may require many years, ochronosis is manifested by a patch of light brown or slate gray pigment in the sclerae on either side of the corneal limbus (Fig. 2) and by a bluish discoloration of the external ears. Those portions of the ear cartilages containing pigment do not transmit light and feel thick and inelastic. The nasal cartilages and superficial muscle tendons of the hands exhibit a bluish discoloration, and brown pigmentation is seen in the skin particularly of the face, axillary folds, thenar and hypothenar eminences, and in the nails. The perspiration may be black or brown and the cerumen similarly deepened in color.

On gross examination at postmortem the pigment appears black or blue-black. It is present in fibrous tissue, fibrocartilage, cartilage, tendon, epidermis, connective tissue, atheromatous and calcified areas, albuminous masses and concretions. On microscopic examination the pigment is yellow, granular or homogeneous. It may be deposited either inside or outside of cells. It may be so plentiful as to obscure the cell architecture, particularly in degenerate cells.

Kleinschmidt[5] and Puhr[6] carried out thorough microchemical studies on ochronotic pigment.

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It has been demonstrated that it does not take iron or fat stains. It is not increased in intensity when stained with silver nitrate according to the method of Levaditi and it is bleached when treated for twenty-four hours with hydrogen peroxide. It is slightly soluble in hydrochloric acid and quite soluble in alkali. By staining technics and chemical analyses the pigment has been shown to resemble melanin.[5–8] There is no known stain specific for ochronotic pigment.

Shortly after ochronosis was recognized as a complication of alcaptonuria,[9,10] instances of its appearance following the long-continued application of phenol dressings to leg ulcers[11,12]

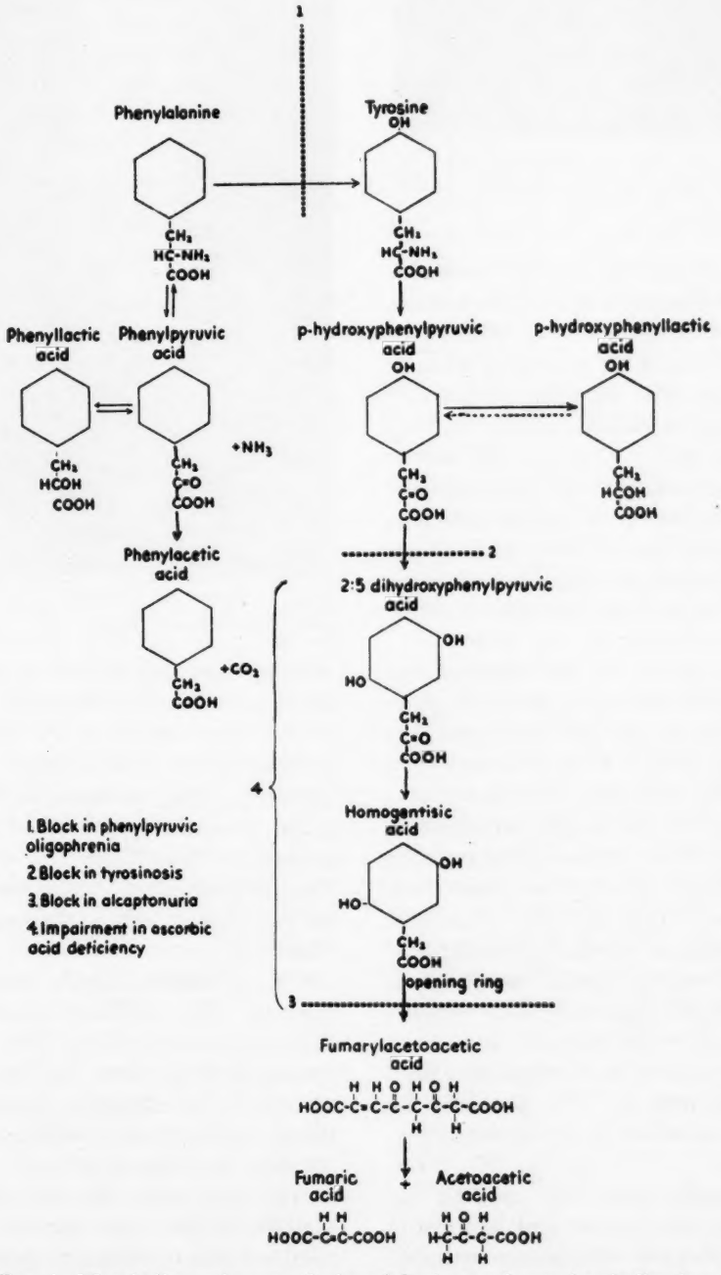

FIG. 1. Metabolic pathways of phenylalanine and tryosine indicating site of block in alcaptonuria, phenylpyruvic oligophrenia, tyrosinosis and ascorbic acid deficiency. Modified after Peters, J. P. and Van Slyke, D. D. Quantitative Clinical Chemistry. Interpretations, vol. I, 2nd ed., p. 766. Baltimore, 1946. The steps in the oxidation of homogentisic acid conform with recent studies referred to in the body of this paper.

FIG. 2. Case II. Illustrates the presence of a patch of light brown pigment in the scleras on either side of the corneal limbus.

and in association with melanuria were reported.[13,14] The intensity of pigmentation following prolonged application of phenol to ulcers has been observed to recede gradually after the use of phenol has been discontinued.[15] In contrast, the pigmentation which accompanies alcaptonuria and melanuria, by virtue of the permanence of the metabolic disturbances, is persistent and progressive. Although ochronosis may follow diverse causes, the findings on postmortem examination are alike[7,15] and histochemical analyses of the pigment give similar reactions.

In spite of the similarity in distribution and appearance of the ochronotic pigments their origin is distinct. Alcaptonuric pigment is a derivative of homogentisic acid whereas melanotic pigment is derived from 3,4-dihydroxyphenylalanine ("dopa"). Although homogentisic acid and "dopa" are both derivatives of tyrosine oxidation, the formation of pigment from these compounds differs. The enzyme tyrosinase catalyzes the oxidation of "dopa" to melanin,[16] but whether the polymerization of homogentisic acid to the ochronotic pigment also requires enzymatic reactions is not known. However, there is no reason to believe that tyrosinase would be involved in this process. The pathway by which phenol is converted to ochronotic pigment is unknown.

By the time alcaptonurics reach middle age they generally complain of pain and stiffness in the large joints due to a deforming arthritis. The spine becomes rigid, there is kyphosis in the dorsal region, lumbar lordosis is obliterated or accentuated and the gait is stooping. Motion in the hip, knee and shoulder joints may become limited and painful. The intensity of symptoms

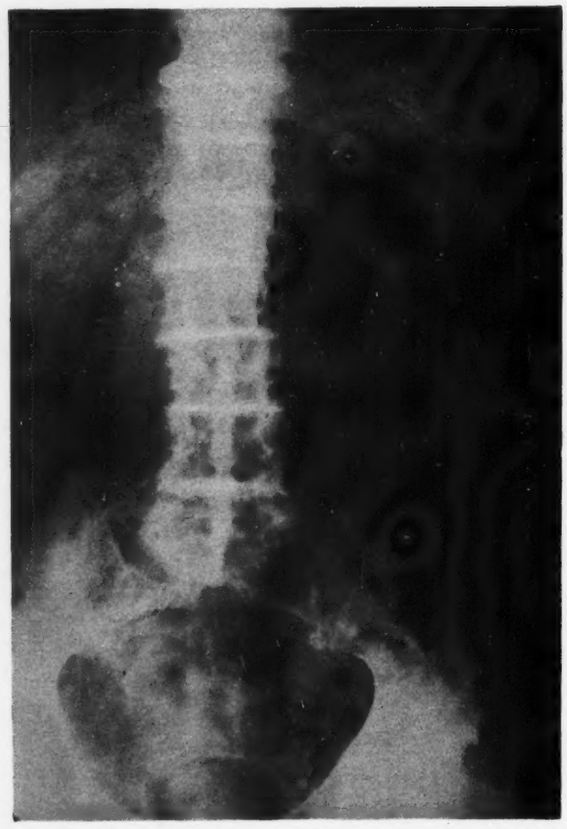

FIG. 3. Note the presence of thin, densely calcified, intervertebral cartilages.

and physical disability varies in different individuals. Of the three patients we have observed two were males in their late fifties exhibiting marked arthritis by roentgenogram, one was totally incapacitated, the other able to walk clumsily but without pain. The third patient, a ninety-four year old woman, first experienced joint symptoms and difficulty in getting about when she was well on in her eighties.

On roentgenographic examination the presence of thin, densely calcified intervertebral discs is characteristic. (Fig. 3.) These become frayed, and in some instances are extruded or resorbed and adjoining vertebral bodies become fused. In addition, degenerative osteo-arthritic changes are apparent in the spine and lumbosacral, sacro-iliac, shoulder, hip and knee joints. Calcific deposits are commonly seen in muscle tendons about the large joints. It is interesting that the smaller joints exhibit little or no alteration.

The relationship between pigmentation and joint changes is not clear since intense pigmentation may be seen postmortem in the absence of

joint changes[17] and arthritis in the presence of only moderate pigmentation.[7]

The incidence of heart disease found postmortem in individuals with ochronosis is relatively high.[14,15,18] There is no specific pathologic finding other than the presence of pigment in areas of degeneration and in the chordae tendinae. Bauer[19] and Kohlmann[20] have reported that stones in the urinary bladder, prostate gland and urethra demonstrated by roentgenograms and at necropsy are not uncommon in alcaptonurics.

From a biochemical viewpoint, alcaptonuric patients have served as a valuable means of studying some aspects of the intermediary metabolic pathway of tyrosine and phenylalanine oxidation. Many compounds which theoretically could be tyrosine oxidation intermediates have been fed to learn whether they augment homogentisic acid excretion. Failure of an increase to occur has been interpreted to mean that the compound is not an intermediate in tyrosine oxidation. However, it should be pointed out that with such feeding experiments it is possible to study only the steps in tyrosine oxidation preceding the formation of homogentisic acid. Misleading results would be obtained if a compound fed, while not a direct intermediate itself, were further metabolized to a compound which normally occurs in tyrosine oxidation, thus resulting in an increase in homogentisic acid excretion. A possible example of this is the observation that 2,5-dihydroxyphenylalanine produces an increase in homogentisic acid excretion when fed to an alcaptonuric.[21] Rather than conclude that this amino acid is a direct intermediate of tyrosine oxidation, an alternate explanation could be, as Neuberger points out,[21] that it is first converted to 2,5-dihydroxyphenylpyruvic acid which in turn is converted to homogentisic acid.

Biochemical *in vitro* studies with animal tissues,[22–25] some of which have involved the use of tyrosine and phenylalanine,[26–28] have supported the concepts derived from these feeding experiments concerning the conversion of phenylalanine and tyrosine to homogentisic acid. In addition they have extended our knowledge concerning the steps involved in the further catabolism of homogentisic acid.

The present concept of the metabolic pathway of phenylalanine and tyrosine is given in Figure 1. Experiments using C^{14} and C^{13} labeled phenylalanine[26] and tyrosine[27,28] with preparations of rat liver have shown that acetoacetic acid is split off as a 4 carbon unit, 2 carbon atoms from the ring and two from the side chain, the remaining 4 carbon atoms of the benzene ring yield fumaric acid, and the original carboxyl carbon of the amino acid is lost as CO_2 in an earlier step. In this way the fate of all of the carbon atoms of tyrosine and phenylalanine has been determined.

The products, acetoacetic acid and fumaric acid, explain the observations that tyrosine is both a ketogenic and glycogenic amino acid. The latter effect is due to the further metabolism of fumaric acid to glucose.

As shown in Figure 1, alcaptonuria is assumed to be a block in the pathway following the formation of homogentisic acid and represents a failure to carry the metabolism through the next step.

There has been disagreement in the past as to whether, in addition to this defect, homogentisic acid itself might not be an abnormal metabolic product, accumulating because of a block in oxidation of some closely related compound. From this point of view homogentisic acid is not on the main pathway of tyrosine oxidation. However, the consensus is that homogentisic acid occurs normally in the catabolism of tyrosine and phenylalanine and this view is well supported by experimental data. Homogentisic acid is, as first shown by Garnier and Voirin,[29] completely metabolized by normal individuals except in very large doses[30] or under certain poorly understood circumstances.[31–36] The work of Katsch and Hurthle[37–39] in patients with depleted glycogen stores and the reported transitory cases of alcaptonuria emphasize this point.[40–49] Some homogentisic acid is excreted by normal adults after ingestion of very large amounts of tyrosine[50] and by animals after large amounts of phenylalanine or of tyrosine.[3,51–54]

The evidence of Dakin[55,56] and others[57] conflicts with the view that homogentisic acid is a product of normal intermediate metabolism. They observed that certain substitute derivatives of phenylalanine and tyrosine whose structures preclude the formation of homogentisic acid are completely catabolized by both alcaptonurics and normal individuals. These observations led Dakin to conclude that "the inference certainly appears probable that alcaptonuria represents a condition in which the formation of homogentisic acid is abnormal as well as the failure to effect its catabolism when

formed."[55] However, it is possible that these analogues of phenylalanine and tyrosine were metabolized by a different metabolic pathway than the normal one followed by phenylalanine and tyrosine. Grutterink and Hymans van den Bergh[33] also believed that homogentisic acid is not a product of normal intermediate metabolism. They observed some individuals with severe diabetes mellitus and some with liver disease with limited capacity to metabolize homogentisic acid. When these individuals were fed tyrosine in an amount which should theoretically have produced sufficient homogentisic acid to exceed their proven limited ability to metabolize it, they did not excrete any of the acid. This line of evidence assumes that in these diseases the ability to form homogentisic acid remains unimpaired. This assumption is unlikely since it has been demonstrated that in liver disease the oxidation of tyrosine[58] and of p-hydroxyphenylpyruvic acid[59] is impaired. Therefore, it is not certain that at any given time sufficient homogentisic acid would have been formed from the tyrosine to exceed the capacity to catabolize it.

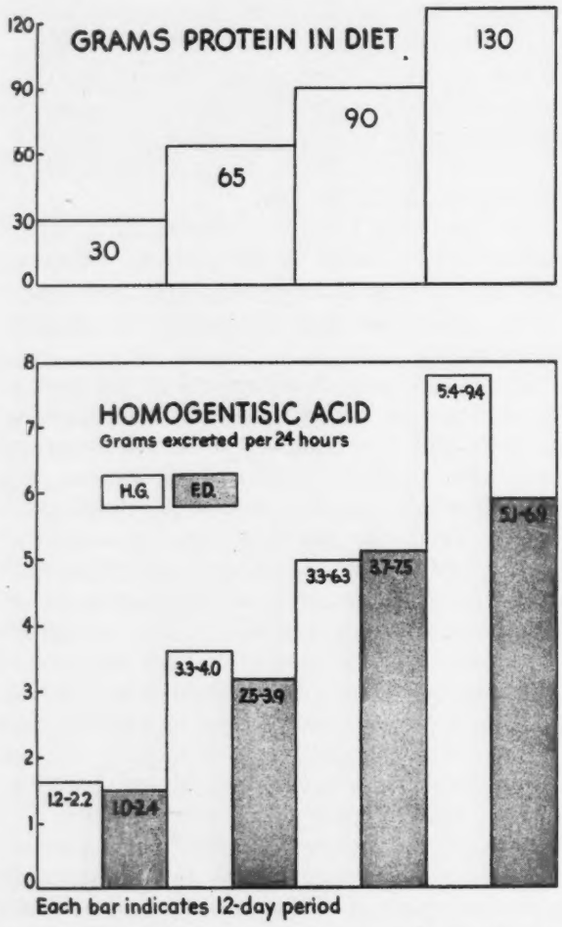

FIG. 4. Comparison between the rate of excretion of homogentisic acid in Case I and Case II when the protein content of the diet was varied. The height of each bar indicates the average daily rate of homogentisic acid excretion for each period; the values beneath the top of each bar, the range of homogentisic acid excretion.

There have been several attempts to demonstrate a difference between the blood of normal and alcaptonuric individuals to explain the latter's inability to metabolize compounds with hydroxyl groups attached to the 2–5 positions of the benzene ring. Homogentisic acid has such a configuration. (Fig. 1.) Gross arrived at the conclusion that an enzyme capable of breaking down homogentisic acid to acetone is present in normal human, dog and rabbit serum, and is absent in alcaptonurics.[60] It must be mentioned, however, that none of the obvious control reactions were carried out. Katsch and Stern[61] in 1926 ascribed the difference between the effect of normal and alcaptonuric sera on homogentisic acid to the presence of an oxidation-inhibiting substance in the latter serum, and believed they had demonstrated the formation in an acid medium of a colorless oxidation product of homogentisic acid which they named "oxyalkapton." Lanyar and Lieb[62] were unable to confirm the work of either Gross or Katsch and Stern and showed, furthermore, what is now well recognized,[63–65] that the oxidation of homogentisic acid occurs slowly when the medium is neutral and increases as it becomes more alkaline. There was no difference between normal and alcaptonuric sera.

Wolkow and Bauman[66] in 1891 were the first to demonstrate that ingestion of tyrosine by an alcaptonuric is followed by an increase in homogentisic acid excretion. Shortly thereafter Falta and Langstein[31] demonstrated that phenylalanine also caused an increase in homogentisic acid excretion. Langstein and Meyer established the usefulness of observing the H/N ratio:

$$\frac{\text{Homogentisic acid (gm.) excreted in 24 hours urine} \times 100}{\text{Nitrogen (gm.) in 24 hour urine}}$$

in metabolic studies in alcaptonuria.[67] The ratio is constant even though the amount of protein in the diet is varied, but changes when the

protein is rich or poor in tyrosine or phenylalanine. Falta showed that homogentisic acid excretion in alcaptonuria varies directly with the estimated amount of tyrosine and phenylalanine in the diet and suggested that the variation in homogentisic acid excretion in some of the studies published up until then was due to lack of adequate control of protein intake.[68] Neubauer emphasized that the rate of excretion of homogentisic acid depends upon the amount of protein catabolized rather than the amount ingested.[69] Evidence in support of this view is the excretion of small amounts of homogentisic acid even during fasting[32,70–73] and the increased rate of excretion during fever[74,75] and following the ingestion of thyroid extract.[32,74]

METABOLIC STUDIES IN TWO CASES

During 1939–1940 the opportunity to conduct metabolic studies in two middle aged alcaptonuric male patients (Case I, H. G. and Case II, F. D.) presented itself. These were carried out with the following purposes in mind: (1) to relate the excretion of homogentisic acid to the quantity of protein consumed and to compare the rate of excretion in these subjects under comparable conditions; (2) to test the effectiveness of the D- and L- forms of phenylalanine in increasing homogentisic acid excretion, and (3) to attempt to decrease the rate of excretion of homogentisic acid with various substances.

Diets of known constant carbohydrate, fat and protein content were served during each

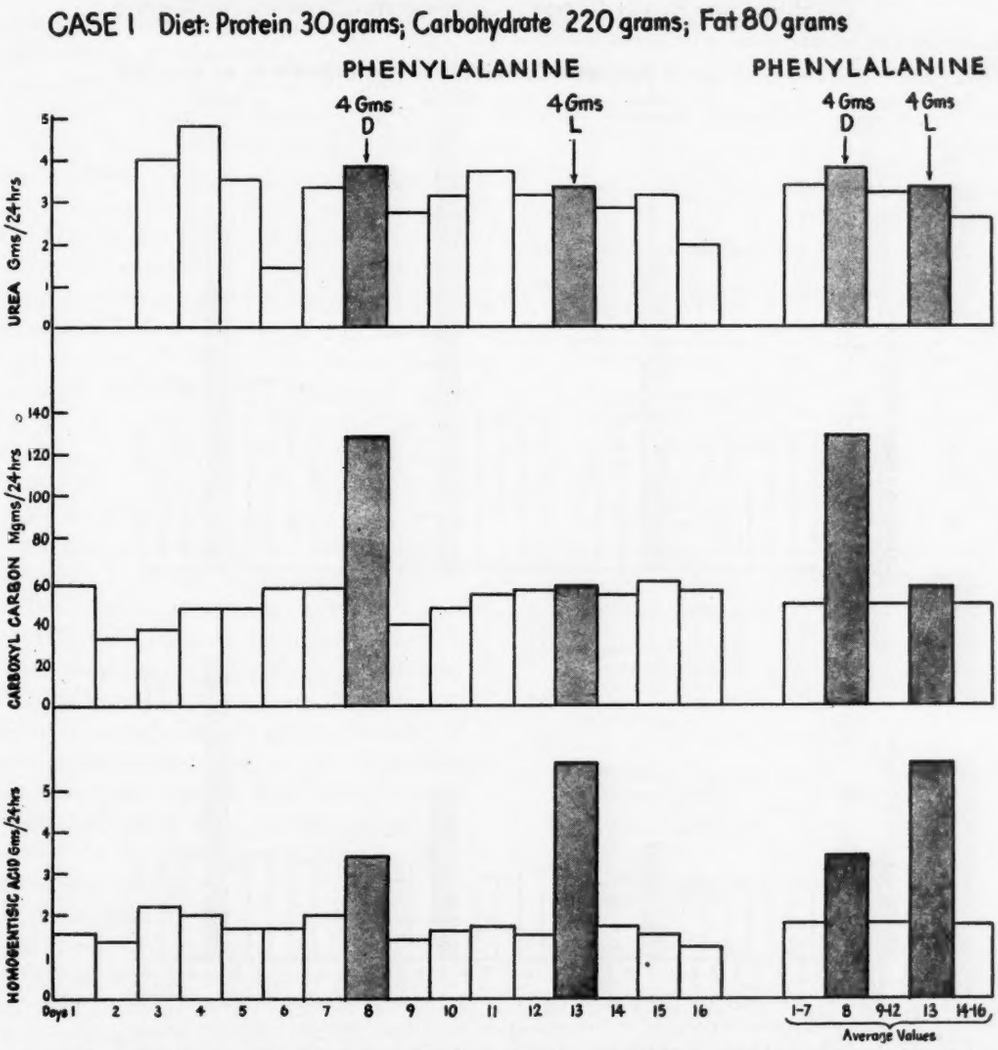

FIG. 5. Summarizes the studies in Case I dealing with the metabolism of D– and L–phenylalanine. The daily urinary excretions of homogentisic acid, carboxyl carbon (alpha amino acid), and urea nitrogen were measured for several days before and after the amino acids were administered.

phase of the studies. The patients ate only the food which was served and in nearly every instance consumed all of it. Whenever a change in the protein content of the diet was made, three to five days were allowed for metabolic readjustment before the control period of the next phase of the study was begun. Urine was collected in a dark bottle during the twenty-four-hour period from 9 A.M. to 9 A.M. Twenty-five cc. of 1 N H_2SO_4 and a few drops of toluene were added to the bottle at the start of each day's collection. During the studies with D- and L-phenylalanine each day's urine volume was collected in two twelve-hour periods, from 9 A.M. to 9 P.M. and from 9 P.M. to 9 A.M. Homogentisic acid in the urine was determined by the method of Lieb and Lanyer[76] and the daily output was expressed in grams. The daily amino acid[77]* and urea[78] excretion in the urine was determined during certain periods of the studies.

1. *The Influence of the Protein Content of the Diet on the Rate of Excretion of Homogentisic Acid.* Both patients were maintained on a diet containing 180 to 220 gm. of carbohydrate, 80 gm. of fat and, during periods of two to three weeks each, 30, 65, 90 and 130 gm. of protein. Homogentisic acid excretion varied directly with the protein content of the diet to practically the same degree in both patients. (Fig. 4.) On the 130 gm. protein diet Case II (F. D.) failed to eat all the food served and excreted less homogentisic acid than Case I (H. G.) during this period.

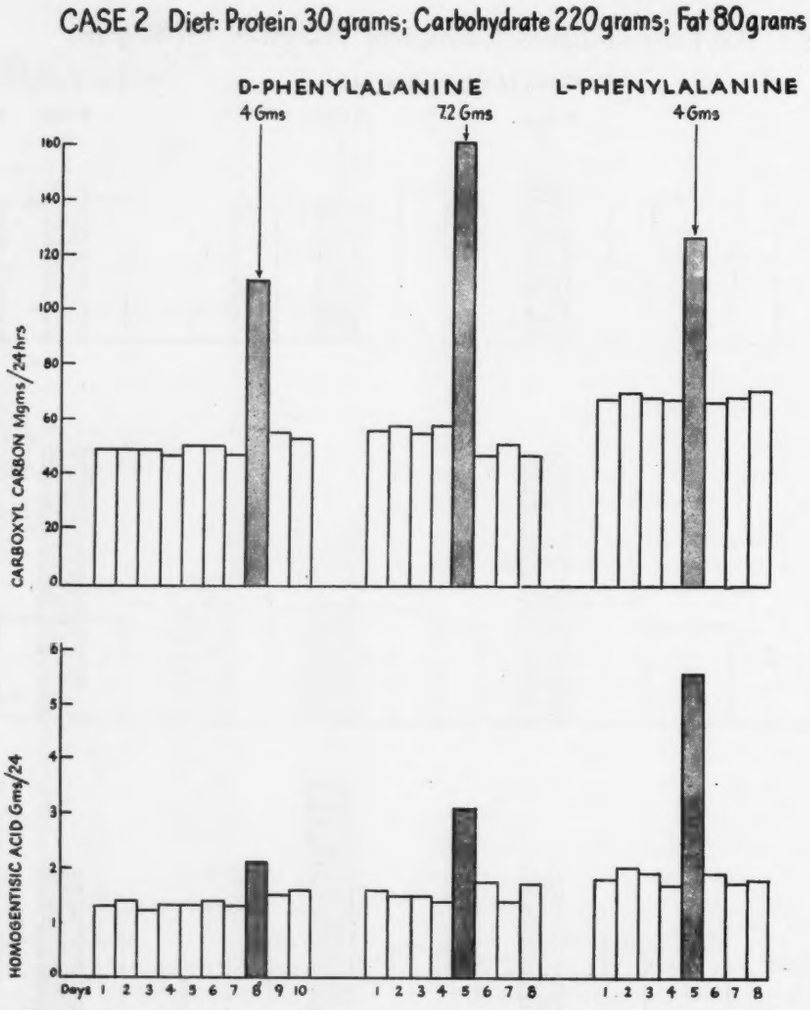

FIG. 6. Summarizes the studies on D– and L–phenylalanine in Case II. The daily urinary excretion of homogentisic acid and carboxyl carbon (alpha amino acid) were measured for several days before and after the amino acids were administered.

* We are grateful to Dr. Donald D. Van Slyke for making available to us this method prior to its publication.

2. *Comparison of the Metabolism of D- and L-Phenylalanine.* This study was carried out while the patients were on a diet containing 30 gm. protein, 220 gm. carbohydrate and 80 gm. fat. Four 1 gm. doses of D- or L-phenylalanine dissolved in 50 cc. of physiologic saline solution were given intravenously at four-hour intervals. Both patients excreted 92 per cent (3.7 gm.) of L-phenylalanine as homogentisic acid and Case I (H. G.) excreted 2.5 per cent (0.1 gm.) and Case II (F. D.) 20 per cent (0.8 gm.) as an amino acid which was not identified. (Figs. 5 and 6). When D-phenylalanine was administered, Case I (H. G.) converted 40 per cent (1.6 gm.) and Case II (F. D.) 20 per cent (0.8 gm.) to homogentisic acid; 35 per cent (1.4 gm.) and 45 per cent (1.8 gm.) could be accounted for as amino acid in Case I (H. G.) and II (F. D.), respectively (Figs. 5 and 6). In another experiment when Case II (F. D.) received intravenously over a period of six hours 7.2 gm. of D-phenylalanine diluted to 2 per cent in physiologic saline solution, he converted 25 per cent (1.8 gm.) to homogentisic acid and 21 per cent (1.5 gm.) was excreted in the urine as amino acid. (Fig. 6.) The proportion converted to homogentisic acid was virtually the same as when he received 4 gm. of D-phenylalanine. In both patients L-phenylalanine was converted not only to a greater degree to homogentisic acid than D-phenylalanine (Table I) but also more rapidly. Nearly all of the L-phenylalanine could be accounted for as homogentisic acid within four hours of the last injection whereas the conversion of D-phenylalanine to homogentisic acid required up to twenty-four hours.

3. *Study of the Effect of Various Substances on the Rate of Excretion of Homogentisic Acid.* Various substances which might conceivably participate in enzyme systems were administered in large doses to learn whether they reduced the rate of excretion of homogentisic acid while the patients were on a diet of constant composition. These included all the vitamins which were available to 1939–1940, brewers' yeast, crude and purified liver extracts, insulin, adrenal cortical extract and tyrosinase. All of the studies were carried out in patient H. G. (Case I) except those with vitamin K_1 and sodium glycocholate, in which patient F. D. (Case II) was also included. Only after brewers' yeast was a change in excretion of homogentisic acid noted, and this was an increase due, no doubt, to the high protein content of the yeast.

Water Soluble Vitamins. Components of vitamin B complex and brewers' yeast were administered as indicated in Figure 7 and vitamin C as indicated in Figure 8.

Fat Soluble Vitamins. (Diet: 90 gm. protein, 220 gm. carbohydrate and 80 gm. fat.) Vitamin D in the form of viosterol was fed in divided doses for three days, starting with 10,000 U.S.P. units and ending with 28,000 units. Vitamins D and A (11,000 to 22,000 U.S.P. units of vitamin D and 62,000 to 160,000 U.S.P. units of vitamin A) were fed in the form of oleum percomorphum for two days. Both patients received 50 mg. vitamin K_1 and 1.2 gm. sodium glycocholate for four days and 10 mg. vitamin K_1 intramuscularly and 1.2 gm. sodium glycocholate orally for six days.

TABLE I

COMPARISON BETWEEN THE METABOLISM OF L-PHENYLALANINE AND D-PHENYLALANINE IN CASES I AND II (H.G. AND F.D.)

	Patients H.G	Patients F.D.	F.D.
	L-phenylalanine		
(1) L-phenylalanine injected (gm.)	4.0	4.0	...
(2) L-phenylalanine excreted as homogentisic acid (gm.)	3.7	3.7	...
(3) L-phenylalanine excreted as amino acid (gm.)	0.1	0.8	...
L-phenylalanine accounted for in urine (gm.) (2+3)	3.8	4.5	...
L-phenylalanine accounted for (%)	95	112	...
L-phenylalanine excreted as homogentisic acid (%)	92	92	...
	D-phenylalanine		
(1) D-phenylalanine injected (gm.)	4.0	4.0	7.2
(2) D-phenylalanine excreted as homogentisic acid (gm.)	1.6	0.8	1.8
(3) D-phenylalanine excreted as amino acid (gm.)	1.4	1.8	1.5
D-phenylalanine accounted for in urine (gm.) (2+3)	3.0	2.6	3.3
D-phenylalanine accounted for (%)	75	65	46
D-phenylalanine excreted as homogentisic acid (%)	40	20	25

Insulin and Adrenal Cortical Extract. (Diet: 65 gm. protein, 220 gm. carbohydrate and 80 gm. fat.) The initial insulin dose was 13 units (5-3-5 one-half hour before each meal) and it was gradually increased to 25 units a day

(10-5-10) over a period of twelve days; 10 to 20 cc. of adrenal cortical extract (Upjohn) were injected intramuscularly daily for five days.

Liver Extracts. (Diet: 90 gm. protein, 220 gm. carbohydrate and 80 gm. fat.) The following liver preparations were injected intramuscularly: Campolon 10 cc. (30 units) for two days; concentrated liver extract 2 cc. (30 units) for four days, and 4 cc. (60 units) for five days; and a crude liver extract (Rockefeller Institute) 10 cc. a day for four days.

Tyrosinase. Two subcutaneous injections of 1,500 units (1.2 cc.) of tyrosinase were given on alternate days to patient H. G. (Case 1) while on a diet of 90 gm. protein, 220 gm. carbohydrate and 80 gm. fat. Each injection was followed by an elevation of temperature within eight hours. The fever reached a maximum of 102.8°c. and gradually subsided within twelve hours. The rate of excretion of homogentisic acid did not increase during the febrile periods nor did it decrease significantly during the

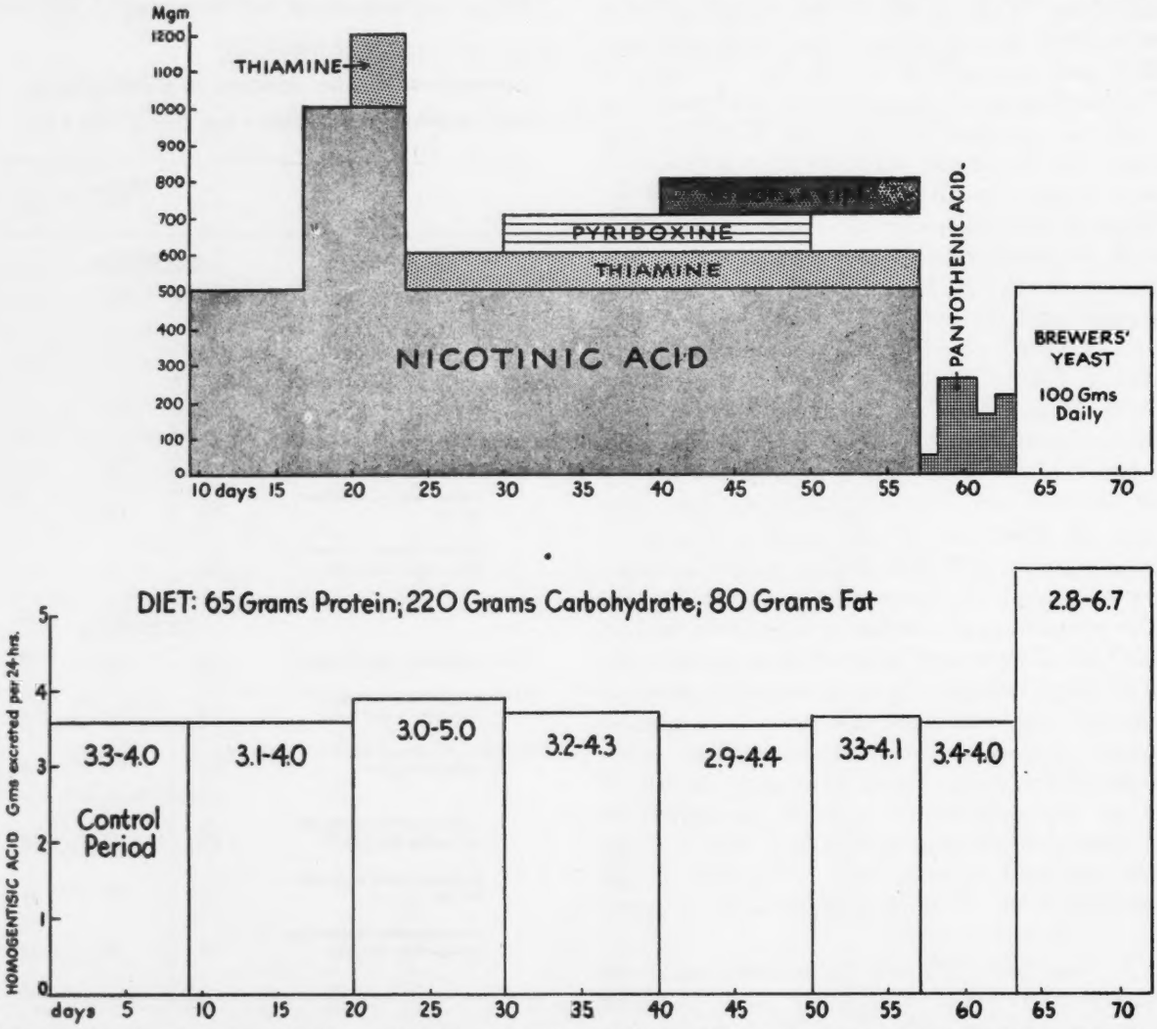

FIG. 7. The vitamins were administered as follows: nicotinic acid, five 100 mg. doses by mouth daily except from the twentieth through the twenty-third day when five 100 mg. doses were also given intravenously and from the seventeenth through the nineteenth day when five 200 mg. doses were given orally; thiamine chloride, five 20 mg. doses orally from the twentieth through the fifty-seventh day except from the twentieth through the twenty-third day when four 25 mg. doses were also administered intravenously; pyridoxine, two 25 mg. doses subcutaneously from the thirtieth through the thirty-seventh day, intravenously from the thirty-eighth through the forty-third day, orally from the forty-fourth through the fiftieth day; riboflavin, four 25 mg. doses intramuscularly from the forty-first through the forty-third day, orally from the forty-fourth through the fiftieth day, intravenously from the fifty-first through the fifty-seventh day; D-calcium pantothenate, two equally divided doses orally and intravenously from the fifty-eighth through the sixty-second day; brewers' yeast, five 20 gm. oral doses from the sixty-third through the seventy-third day.

afebrile periods between injections nor during several days following the last injection.

Fifteen minutes following the subcutaneous injection of 3,000 units (2.4 cc.) of tyrosinase in 50 cc. of physiologic saline a severe febrile reaction occurred. The temperature reached a maximum of 105.6°C. within forty minutes and gradually subsided during the next ten hours. The patient was incontinent of urine during the early part of this febrile period. During the following few days he remained afebrile and the rate of excretion of homogentisic acid remained unaltered.

The arterial pressure fell during the period of injection of tyrosinase from its usual level of 180/120 to as low as 108/72, and returned four days following the last injection. At each site of injection of tyrosinase a swollen, tender, indurated area with dusky blue discoloration of the skin persisted for many days. The regional lymph nodes were not enlarged.

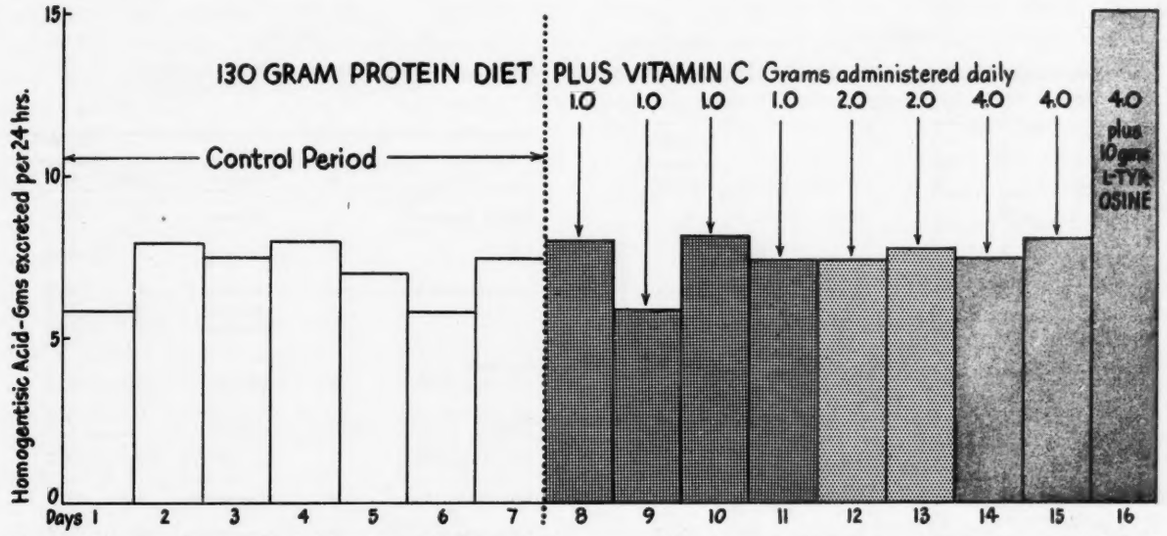

FIG. 8. Vitamin C was administered as follows: eighth through the eleventh day, 1 gm. dose orally; twelfth and thirteenth days, four 0.5 gm. doses orally; fourteenth, fifteenth and sixteenth days, four 1 gm. doses, two orally and two intravenously. In the morning of the sixteenth day a single 10 gm. dose of L-tyrosine was also given by mouth.

COMMENTS

It is apparent that the daily excretion of homogentisic acid varied with the protein content of the diet and that the metabolic disturbance was of the same intensity in both patients. (Fig. 4.) This is not unexpected since they were both of approximately the same age, height and weight.

The previous experiments on the effect of ingestion of tyrosine and phenylalanine on the rate of excretion of homogentisic acid by alcaptonurics indicated that the conversion of these amino acids to homogentisic acid is usually greater when they are administered in divided doses and the naturally occurring levo-rotatory amino acids usually yield more than the racemic mixture. (Tables II and III.) In the single experiment in which 1.5 gm. of L-phenylalanine (natural form) and 1 gm. of D-phenylalanine (unnatural form) were fed to a patient, much more of the natural form was converted to homogentisic acid (Table III, Abderhalden[79]). However, the small amount of D-phenylalanine and the apparent difficulty in maintaining the patient on a controlled diet make the results uncertain. Different observers have reported a considerable difference between the effect of feeding racemic mixtures and natural isomers of both amino acids on the rate of excretion of homogentisic acid. (Tables II and III.) This may depend upon individual difference in rate of absorption of the amino acids, their incomplete absorption or partial destruction by the intestinal flora. In our studies these difficulties were excluded and a more exact comparison of conversion of these isomers to homogentisic acid was made possible by intravenous administration of L- and D-phenylalanine in divided doses. Both patients converted nearly all of the L-phenylalanine and less than 50 per cent of D-phenylalanine to homogentisic acid. (Table I.) The recent observation that 85 to 94 per cent of L-phenylalanine administered intravenously to a young alcaptonuric was con-

verted to homogentisic acid agrees with our results.[21]

Lanyar noted complete conversion into homogentisic acid of moderate amounts of L-phenylalanine and L-tyrosine administered orally in divided doses to an alcaptonuric whereas only 40 to 45 per cent of D-tyrosine and 68 per cent of D,L-tyrosine (racemic form) fed similarly were accounted for as homogentisic acid.[80] It has become evident that the difference between the rate of conversion of the L forms and racemic mixtures of these amino acids to homogentisic acid is due to the incomplete conversion of the D-components.

The difference in the rate of conversion of D-phenylalanine in Case I (H. G.) and Case II (F. D.) may possibly be due to an inherent difference in metabolic capacity for D-phenylalanine similar to that reported in normals.[81]

Attempts to reduce the rate of excretion of homogentisic acid by alcaptonurics have varied with the views taken as to its cause. Since the early failure with intestinal disinfectants and cathartics,[30,70,82–84] substances designed to inhibit its formation[85,86] and to oxidize it[32,74,87–92] have been administered without success. It seems logical to infer that a defect in some enzyme system essential for the breakdown of homogentisic acid is transmitted because a definite hereditary incidence has been established in alcaptonuria.[93]

Although vitamins are known to be components of co-factors required for many enzyme reactions, giving vitamins or vitamin-rich substances did not alter the excretion of homogentisic acid in our patients. There was no

TABLE II

EXPERIMENTS ON TYROSINE IN PATIENTS WITH ALCAPTONURIA

Author	Patient No.	Type of Tyrosine	Grams	Excreted as Homogentisic Acid (%)
Kraske & Baumann[108]	1	Racemic	12.5	93
Wolkow & Baumann[3]	1	Racemic	12.5	75
	1	Racemic	10.0	69
	1	Racemic	11.5	83
Embden[30]	2	Racemic	15.0	33
Mittelbach[70]	3	Racemic	7.5*	92
Langstein & Meyer[67]	4	Racemic	8.6	61
	4	Racemic	10.0*	59
Schumm[51]	5	Racemic	6.0	86
Grutterink et al.[33]	6	Racemic	10.0	80
Blum[109]	7	Racemic	5.0	64
Soderbergh[85]	8	Racemic	15.0	55
Gibson & Howard[110]	9	Racemic	2.0*	45
Koopman[32]	10	Racemic	15.0*	27
Lanyar[80]	16	Racemic	4.0	68
Abderhalden et al.[79]	11	Levo-rotatory	3.0	23
	11	Levo-rotatory	5.0	23
Neubauer[111]	12	Levo-rotatory	5.0*	38
Fromherz[112]	13	Levo-rotatory	9.0	85
Blum[109]	7	Levo-rotatory	5.0	56
Soderbergh[85]	8	Levo-rotatory	15.0	99
	8	Levo-rotatory	15.0	93
Katsch[71]	14	Levo-rotatory	0.5*	16
Sealock et al.[95]	15	Levo-rotatory	10.0	88
Lanyar[80]	16	Levo-rotatory	4.0	93
White et al.[86]	17	Levo-rotatory	8.0	53

*Indicates single dose; otherwise fed in divided doses.

TABLE III

EXPERIMENTS ON PHENYLALANINE IN PATIENTS WITH ALCAPTONURIA

Author	Patient No.	Type of Phenylalanine	Grams	Excreted as Homogentisic Acid (%)
Falta & Langstein[31]	1	Racemic	4*	38
Falta[68]	2	Racemic	4	86.5
Abderhalden et al.[79]	3	Racemic	8	78.0
	4	Racemic	3	22.0
	4	Racemic	5	30.0
Grutterink & van den Bergh[33]	5	Racemic	10	76.0
Fromherz[112]	6	Racemic	7	37.0
Falta & Langstein[31]	1	Levo-rotatory	5	89.3
Abderhalden et al.[79]	4	Levo-rotatory	1.5	106.0
Papageorge et al.[113]	7	Levo-rotatory	10	90.0
	7	Levo-rotatory	5	104.0
	7	Levo-rotatory	10*	50.0
	7	Levo-rotatory	15*	72.0
	8	Levo-rotatory	10*	64.0
	8	Levo-rotatory	10*	28.0
Steele et al.[114]	9	Levo-rotatory	4†	92.0
	10	Levo-rotatory	4†	92.0
Lanyar[80]	11	Levo-rotatory	5†	106.2
	11	Levo-rotatory	4†	106.6
Abderhalden et al.[79]	4	Dextro-rotatory	1	56.0
Steele et al.[114]	9	Dextro-rotatory	4†	40.0
	10	Dextro-rotatory	7.2†	25.0
Lanyar[80]	11	Dextro-rotatory	5†	38.6
	11	Dextro-rotatory	4†	43.6
	11	Dextro-rotatory	3†	44.8
Neuberger et al.[21]	12	Dextro-rotatory	3‡	85.0 to 94.0

*Indicates a single dose; otherwise fed in divided doses.
†Administered intravenously in divided doses.
‡Administered intravenously in single doses.

clinical evidence that a vitamin deficiency was present.

While no co-factor needed for the enzyme system oxidizing homogentisic acid has been isolated, recent work suggests that one exists.[94] The alcaptonuric might lack the ability to synthesize the needed co-factor or might be unable to synthesize the enzyme itself.

Of the vitamins only ascorbic acid had been tested previously and reports concerning its efficacy had conflicted. Monsonyi[91] reported that intravenous injections of vitamin C decrease the excretion of homogentisic acid whereas Diaz and his co-workers reported that ingested vitamin C is without effect.[92] The latter group did not try the effect of intravenous administration of vitamin C. In our studies vitamin C in large doses by mouth and by vein did not alter the rate of homogentisic acid excretion.[95] A possible relation between homogentisic acid and vitamin C was excluded when a single dose of 10 gm. of L-tyrosine was given by mouth in the morning and 4 one-gram doses of vitamin C were administered, two orally and two intravenously during the day without altering the expected rate of conversion of L-tyrosine to homogentisic acid; 88 per cent of the tyrosine was recovered as homogentisic acid in the urine. (Fig. 8.) The recent studies by Neuberger and his co-workers[21] confirm the ineffectiveness of ascorbic acid in altering homogentisic acid excretion and indicate, as our studies do, that its value lies only in its ability to prevent the urine from turning dark readily since it is a stronger reducing agent than homogentisic acid.

The absence of effect of vitamin C in inherited alcaptonuria is particularly interesting since it is effective in alcaptonuria induced experimentally in guinea pigs on a diet deficient in ascorbic acid and rich in phenylalanine and tyrosine.[96] In addition to homogentisic acid the guinea pigs excrete p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acids in the urine. (Fig. 1.) Premature and full term infants may also exhibit a disturbance in metabolism of tyrosine and phenylalanine which is prevented by ascorbic acid[97] or ACTH.[98] When fed a diet rich in protein or large amounts of phenylalanine or tyrosine, their urine contains p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids but no homogentisic acid. The fact that ascorbic acid prevents these induced metabolic derangements and not the inherited type of alcaptonuria indicates that there is a basic difference between them. (Fig. 1.) It suggests that the induced disorders result from overloading of an enzyme system for which ascorbic acid is essential. Recent experiments using animal tissue preparations have demonstrated the importance of ascorbic acid in tyrosine oxidation.[99,100] Effective therapy has not been devised for the other known disturbances of phenylalanine and tyrosine metabolism: phenylpyruvic oligophrenia and tyrosinosis. Phenylpyruvic oligophrenia, which involves only phenylalanine, occurs in certain mentally deficient individuals who excrete phenylpyruvic and phenylacetic acids and phenylalanine.[101,102] (Fig. 1.) Tyrosinosis, of which but one instance has been reported, is believed to be due to inability to oxidize p-hydroxyphenylpyruvic acid, a derivative of tyrosine.[103] It is characterized by excretion in the urine of tyrosine and p-hydroxyphenylpyruvic acid.

There are conflicting reports concerning the therapeutic value of liver extract in the inherited form of alcaptonuria. In 1936 Klein and Bloch reported a sharp fall in urinary homogentisic acid to as little as one-eighth to one-tenth of its usual level within four to six hours following intramuscular injection or ingestion of liver.[92] They expressed homogentisic acid excretion in milligrams per cent but the daily volume of urine was not reported. Others have failed to confirm their results.[86,93,95,101] We did not observe any effect from large intramuscular injections of crude and purified liver extracts.

Our studies on the effect of insulin and adrenal cortical extract on the rate of excretion of homogentisic acid were stimulated by the suggestion that the metabolism of carbohydrate is related to that of homogentisic acid. Mittelbach[70] in 1901 noted a moderate fall in the rate of excretion of homogentisic acid with starvation in an alcaptonuric individual. Two years later Falta and Langstein[31] reported that a severely diabetic patient excreted in the urine nearly half of 4 gm. of homogentisic acid fed while normal individuals excreted none. Clinical studies carried out approximately twenty to thirty years later in alcaptonuric individuals,[37–39,63,72,105,106] severely ill diabetic patients and in patients suffering from marked cachexia of many etiologies[32–36] were believed to demonstrate that carbohydrate is essential both for the formation of homogentisic acid from tyrosine and for its ultimate catabolism. In view of the present day knowledge that multiple nutritional deficiencies

occur in such clinical states, it is questionable whether these studies have clearly established definite metabolic interrelationships.

Although clinical studies on alcaptonuric individuals have contributed much to our knowledge of the intermediate pathway of tyrosine catabolism, it does not seem likely that such studies alone will furnish the details of the still unknown steps. It appears that future progress in this phase of the problem will require the use of simpler *in vitro* systems, such as liver homogenate or liver acetone powder preparations. Recently, Ravdin and Crandall[107] isolated two fractions of a rat liver homogenate preparation, one which catalyzes the oxidation of homogentisic acid to fumarylacetoacetic acid, and another which hydrolyzes fumarylacetoacetic acid to fumaric acid and acetoacetic acid. (Fig. 1.) If homogentisic acid is a normal intermediary compound, the enzyme systems involved in its further oxidation may now be carefully studied. Further studies with this system should show what components are needed for the further oxidation of homogentisic acid, and it should then be possible to find more specifically where the metabolic deficiency exists in alcaptonuria.

CASE REPORTS

CASE 1. H. G., a sixty-five year old white male, was hospitalized on July 14, 1939, because of a painful right hip resulting from fractures in 1929 and 1939. While he was suffering from bronchopneumonia in October, 1939, the medical consultant noted that he voided black urine, that a patch of light brown pigment was deposited in the temporal side of the sclera of each eye and that there was a dusky blue discoloration of the aural cartilages. The patient did not recall having voided black urine previously but a note from another hospital where he was under observation in October, 1938, stated that "although the urine was jet-black it was negative for bile and blood." In 1934 he was treated for diabetes mellitus with dietary regulation by a private physician. He was known to have arterial hypertension since 1931. Iridectomy and cataract extractions from both eyes were performed in 1932 and 1933, respectively, and discission of secondary cataracts in February, 1936.

He had no knowledge of the excretion of dark urine by any member of his family or of discoloration of their eyes and ears. He was not born of a consanguineous marriage. Twice married, he had no children.

He was fairly well developed, well nourished and did not appear ill. There was an old depressed fracture of the frontal bone. Both eyes showed evidence of iridectomy and cataract extractions. There was a small patch of light brown pigment measuring about 3 mm. in diameter in the outer half of the sclera of each eye. The retinal arteries exhibited moderate arteriosclerotic changes. The external ears were slate gray in color and the aural cartilages felt thick and inelastic and were opaque on transillumination. The chest was emphysematous. The heart was moderately enlarged to the left, the rhythm regular and the sounds of good quality. There was a rough systolic murmur over the precordium, loudest at the base. The level of arterial pressure varied between 146/100 and 218/120. The liver and spleen were not enlarged. There was a complete reducible left inguinal hernia. There was marked sclerosis and tortuosity of the arteries of the extremities. The right lower extremity was 2 inches shorter than the left due to an old fracture of the lower one-third of the femur. Motion in the right hip joint was markedly limited in all directions but satisfactory in the left hip joint. There was crepitation in the knee joints on motion. The spine exhibited moderate kyphosis and scoliosis to the right and marked limitation of motion in the lumbosacral region. No other abnormalities were noted.

On x-ray examination the heart was moderately enlarged in all diameters. The aorta was diffusely enlarged and a calcareous plaque was present in the transverse portion of the aortic arch. The lungs were clear.

The long bones of the legs were decalcified, their joint spaces narrowed and there was cystic degeneration and productive osteoarthritic changes in the contiguous articulating surfaces. There was complete absorption of the head of the right femur, a healed fracture in the lower one-third of its shaft, and its neck formed an articulation with the acetabular shelf. There was intense decalcification of the vertebral bodies and osteo-arthritic spurs. The intervertebral discs were markedly narrowed and the remnants densely calcified. There was an extensive old depressed fracture of the frontal bone and calcific deposits in the choroid plexus and in the carotid arteries in the region of the sella turcica. Intra-

venous pyelograms were normal. There were several radiopaque prostatic calculi.

The urine exhibited all of the physical properties and qualitative chemical reactions due to the presence of homogentisic acid described in the introductory section. In addition homogentisic acid was isolated from it. The urine exhibited no other abnormalities except for an occasional trace of albumin. There was no impairment of the ability of the kidneys to concentrate urine or to excrete phenolsulphonthalein.

There were 4.5 to 5 million red blood cells per cu. mm. of blood and 13 to 14 gm. hemoglobin per 100 cc. of blood. The white blood cells numbered 6,000 to 8,000 per cu. mm. of blood and the differential count was normal. The Wassermann and Kahn reactions of the blood were negative. At the end of thirty minutes there was no retention in the serum of bromsulphalein injected intravenously (5 mg./Kg.). The glucose tolerance test was normal. The level of serum calcium was 11 to 12 mg. per cent, phosphorus 2.4 to 3.3 mg. per cent, alkaline phosphatase 2.6 to 4.4 Bodansky units; urea nitrogen 15 mg. per cent; albumin 5.3 gm. per cent and globulin 1.6 gm. per cent. Free hydrochloric acid was present in the gastric contents only after the injection of histamine. The electrocardiographic axis was deviated to the left and there was an occasional ventricular premature contraction.

He enjoyed relatively good health throughout the period of observation. In June, 1941, he was transferred to the New York City Farm Colony on Staten Island for custodial care. He remained well until March 2, 1946, when he developed right hemiparesis and partial aphasia. He gradually lost ground, became bedridden and was transferred to Bellevue Hospital on August 14, 1947, for treatment of gangrene of both heels. The patient was considered too ill for surgery. He failed to respond to conservative treatment and died on August 23, 1947.

At postmortem examination* purplish black pigmentation of the superficial tendons of the forearm could be seen through the skin. The costal cartilages, cartilages of the sternoclavicular joints, laryngeal, thyroid, tracheal and bronchial cartilages were black. The intervertebral discs were thin, frayed and densely black.

The heart weighed 350 gm. The base of the aortic valve was rigid and calcified. There were calcified nodules in the sinuses of Valsalva. The mitral valve ring was densely calcified and the base of the posterior cusp of the mitral valve was also calcified. All of these calcified areas appeared black. In addition, the chordae tendinae were black. The tricuspid and pulmonary artery valves were thin and delicate. The coronary ostia were patent and the coronary arteries were markedly sclerotic and tortuous. Their intima were calcified but not pigmented black. The aorta contained many atherosclerotic and calcified plaques, many of which appeared mottled black. Except for black pigmentation of the bronchial cartilages, the lungs appeared normal. Many black punctate spots were present over the cut surface of the kidney. The prostate was diffusely enlarged and contained black, gritty concretions. The meninges were of a mottled gray-black color. There was marked sclerosis of the cerebral arteries and an aneurysmal dilatation at the junction of the basilar and vertebral arteries. There was an extensive anemic infarct of the basal ganglia on the left.

CASE II. F. D., a 60 year old single white cigar maker of French descent, was transferred from the Columbia University Medical Service, Bellevue Hospital, on June 12, 1940, with the diagnosis of alcaptonuria, ochronosis and alcaptonuric arthritis. He complained of progressive stiffness and pain in the shoulders, hips and knees of many years' duration. Since 1937 he had experienced anorexia, constipation and intermittent epigastric pain not related to meals but associated with gaseous eructations. These symptoms did not increase in severity or frequency. He had lost 10 pounds in weight during the year prior to admission. He had not noticed that his urine turned black but was aware of a bluish discoloration of his ears since the age of twenty-five. None of the members of his immediate family had ever to his knowledge passed dark urine or had discoloration of their eyes, ears or skin. He was not born of a consanguineous marriage.

The patient was fairly well developed and nourished. He walked about slowly and painfully with the aid of canes. There was a brown patch of pigment, about 2 mm. in diameter, in the outer and inner third of the sclera of each eye. The right pupil was round, regular and reacted sluggishly to light and accommodation. There was total loss of vision in the left eye, the result of an old injury. The left pupil was irregu-

* We are indebted to the Pathology Department of the Cornell Surgical Division, Bellevue Hospital for the postmortem report.

lar, fixed to light and accommodation. There were several opacities in the vitreous humor of the left eye and there was atrophy of the optic nerve. The right fundus appeared normal except for tortuous and irregular arteries which gave an increased light reflex. The aural cartilages were thick and inelastic and irregularly opaque to transillumination. The overlying skin was slate-gray in color. Light brown macular patches of pigment measuring 1 to 2 cm. were distributed over the body. The skin of the axillary folds was light blue. There were no abnormal pigment deposits in the mucous membranes or cartilaginous portion of the nasal septum. The mouth, pharynx and neck were normal. The lungs were clear. The heart was not enlarged, the rhythm was regular and there was a harsh systolic murmur over the base. The arterial pressure ranged between 120/80 and 100/70. The liver and spleen were not enlarged. The prostate was firm and moderately enlarged. The genitalia were grossly normal. The muscles of the extremities were atrophic and the arteries sclerotic. The nails of the fingers and toes and tendons of the superficial muscles were not pigmented. Neurologic examination revealed no abnormality. The spine was straight and fixed except for a limited ability to flex and extend the neck. All movements in the shoulder, hip and knee joints were limited.

On x-ray examination the lung fields appeared normal. The teleroentgen shadow of the heart was not enlarged. There were large calcareous plaques in the aortic arch and in the abdominal aorta. There were healed fractures in the posterior axillary portions of the sixth, seventh and eighth ribs on the right side. There were calcific deposits in the choroid plexus, falx cerebri and the intracranial portion of the carotid arteries.

The joint spaces of the shoulders and the left hip were narrowed, the articulating cartilages were eroded and the contiguous articulating surfaces of the bones were sclerosed. There were osteo-arthritic spurs in the knee joints and the joint cartilages were thin and calcified. The intervertebral discs were thin and calcified, the intervertebral spaces markedly narrowed and the contiguous vertebral bodies eburnated. There were osteo-arthritic changes throughout the spine. The sacro-iliac joint spaces were narrowed. Numerous calcific deposits were present in the soft tissues of the arms and legs.

A gastrointestinal series revealed no abnormalities. Electrocardiographic changes were consistent with an old infarction of the anterior wall.

The urine exhibited all of the physical and chemical properties due to the presence of homogentisic acid. Tests of the urine for albumin, bile pigments, blood, acetone and diacetic acid were negative; amounts of urobilin and urobilinogen were not increased; urinary sediment was normal.

There were 4.5 million red blood cells and 8,700 white blood corpuscles per cu. mm. of blood and 13.9 gm. of hemoglobin per 100 cc. of blood. The differential white blood count was normal. The fasting level of sugar in the blood was 74 mg. per cent; urea nitrogen 12.6 mg. per cent; non-protein nitrogen 26.6 mg. per cent; uric acid 2.5 mg. per cent; creatinine 2.2 mg. per cent; total cholesterol 284 mg. per cent; cholesterol esters 200 mg. per cent; calcium 11.4 mg. per cent; phosphorus 3.7 mg. per cent; alkaline phosphatase 3 Bodansky units; total protein 6.6 gm. per cent; albumin 4.0 gm. per cent and globulin 1.6 gm. per cent. The blood Wassermann and Kahn reactions were negative. No significant variations appeared in the laboratory data throughout the patient's hospital stay.

Motion in the knee and hip joints gradually decreased so that by April, 1941, the patient was unable to walk. From then until September, 1942, he was relatively well except for short periods of epigastric distress which were relieved by sodium bicarbonate.

On September 11, 1942, the patient experienced generalized abdominal cramps and diarrhea, and vomited small amounts of bright red blood. The heart sounds and radial pulses became feeble. The systolic level of arterial pressure fell to 90 mm. Hg and the diastolic level could not be elicited. An electrocardiogram taken the following day suggested recent infarction of the posterior wall of the heart. Subsequent serial electrocardiograms indicated rapid healing of this infarction. The arterial pressure gradually rose to 105/90 mm. Hg. A gastrointestinal series performed on November 13, 1941, revealed no abnormalities.

On November 25, 1942, the patient exhibited a moderate degree of congestive heart failure. Serial electrocardiograms revealed progressive decrease in voltage and premature ventricular beats from multiple foci. The patient died suddenly on December 7, 1942.

At necropsy, which was performed five days after death, it was observed that in addition to the areas of pigmentation noted during life, black pigment deposits were present in the tracheal, laryngeal, costal, sternoclavicular and intervertebral cartilages; endocardium, heart valves and valve commissures, chordae tendinae at their points of attachment; atheromatous and calcified areas in the aorta and in concretions in the prostate gland.

The heart was hypertrophied and weighed 400 gm. On section, the myocardium showed in the walls of practically the whole of the left ventricle and the interventricular septum, large, ill-defined, firm whitish patches corresponding to areas of scarring. The endocardium revealed small blackish pigment deposits, especially noticeable at the bases of the aortic cusps in the immediate vicinity of the commissures, in the commissures themselves, in the leaflets of the mitral valves, and at the tips of the chordae tendinae at their points of attachment, and in the cusps and commissures of the pulmonary artery valve. In all of these localities the endocardium was thickened and calcified. The aortic valve cusps were thickened, calcified and slightly retracted. The aorta throughout its extent contained innumerable blackish deposits in areas of thickening and calcification. The coronary arteries were markedly calcified. Their lumina were greatly narrowed and in several places practically occluded. The right coronary artery was more extensively involved than the left. Its lumen was almost completely closed. No pigmentation was noted in any part of the coronary artery system.

The prostate gland was normal in size. On section it showed many minute, jet black concretions.

The intervertebral discs were narrowed, marbled in brownish black, and calcified.

Except for the presence of 800 cc. of clear fluid in the right pleural cavity and 400 cc. in the left, 1,000 cc. in the abdomen and 100 cc. in the pericardial sac, the rest of the body, including the brain, showed nothing of note in the present connection.

On histologic examination the muscle tissues of the myocardium were extensively hyalinized. In some instances, at the poles of the intracellular nuclei, there were deposits of pigment that were roughly triangular in shape with the bases toward the nuclei. The pigment was coarsely granular and brownish yellow. It did not respond to stains for iron and seemed to be identical with the pigment found in the same localities in brown atrophy of the heart. Scattered through the hyalinized areas of myocardium were collections of finely granular brownish black pigment which responded to Fontana's stain for melanin. In other places there were patches of finely granular or even amorphous material which stained bluish with hematoxylin and eosin and appeared to be calcium. The papillary muscles were likewise extensively hyalinized and sprinkled with blackish pigment which also stained by the method of Fontana. The fibroendothelial valvular prolongations were thickened, almost completely hyalinized, and revealed clumps of brownish black, finely granular pigment both in their substance and at the commissures, but no deposits of calcium.

The sternoclavicular, costochondral and intervertebral cartilages showed large and small clumps of coarsely granular, brownish black pigment lying in an otherwise normal matrix. The cartilages of other joints were not examined.

The prostate gland showed a moderate degree of fibromuscular hyperplasia. Scattered through the fibromuscular stroma were groups of acini which were lined by epithelial cells or were partially filled by desquamated cells. In the cytoplasm of many of these cells were deposits of coarsely granular, brownish black pigment. In the fibromuscular tissues in the immediate vicinity of the acini were numerous nucleated cylindrical cells in the cytoplasm of which were dense collections of brownish black, coarsely granular pigment. The acini, in many instances, were occupied by corpora amylaceae. Some of these were concentrically laminated, stained fairly pinkish and were free from pigment. The great majority showed no laminations at all, were rounded or oval in outline and were made up practically completely of finely or coarsely granular, blackish brown pigment, arranged diffusely or in clump-like formations. The reaction to Fontana's stain for melanin was positive. None of the corpora amylaceae were calcified.

The epithelial cells lining some of the distal and proximal convoluted tubules of the kidneys contained coarsely granular, brownish yellow pigment lying in the remains of the cytoplasm, only occasionally overflowing the nucleus. The majority of the collecting tubules were pigmented. The cytoplasm of the lining epithelial cells was likewise crowded by yellowish brown

pigment granules. In both the convoluted and collecting tubules the epithelial cells were in an advanced state of disintegration and many of them were desquamated. The pigment in the tubules reacted to Fontana's stain for melanin. In none of the tubules were there any signs of calcification.

The cytoplasm of some of the nerve fibers in the posterior lobe of the pituitary contained small collections of brownish black pigment granules.

CASE III. E. L., a ninety-four year old San Domingo woman, was transferred from City Hospital in October, 1944, for chronic care with the diagnosis of alcaptonuria, ochronosis and generalized arteriosclerosis. She had been suffering from pain and stiffness in the knees and right shoulder and increasing difficulty in walking since 1937. She first noticed darkening of her eyes in 1941 and dark discoloration of her urine in 1943. None of the members of her immediate family had ever, as far as she knew, passed dark urine or had discoloration of the eyes, ears or skin.

The patient came to the United States at the age of seventy-four. She had not been ill in the past except for malaria at the age of fifty and underwent a womb operation at the age of eighty.

She was of slight build, unable to stand erect because of pain and stiffness in the knees. The skin was thin and dark-colored. The sclerae were blue and contained light brown patches of pigmentation measuring 2 to 3 mm. in diameter on either side of the corneal limbus. The external ears exhibited intense bluish discoloration and their cartilages felt thick and inelastic and were opaque on transillumination. Bluish discoloration was noted in the superficial tendons of the hands and feet and in the nasal cartilages. The lungs were clear. The heart was moderately enlarged to the left, the rhythm was regular, blood pressure level ranged from 210/100 to 150/60. There was a harsh systolic murmur over the apex and base of the heart. No abnormalities were noted on examination of the abdomen. There was marked crepitation and limitation of motion in the knee, hip, right shoulder and elbow joints; kyphoscoliosis of the thoracic vertebrae and obliteration of lumbar lordosis. The palpable arteries in the upper and lower extremities were sclerotic. No other abnormalities were noted.

X-ray examination of the vertebral column revealed marked lipping of the vertebral bodies, narrowing of the intervertebral spaces, calcification of flattened and partially extruded intervertebral discs, calcification between the last dorsal and first lumbar vertebrae, scoliosis of the lumbar spine to the left and marked osteoarthritic changes of the sacroiliac joints. The joint space of the right hip was narrow. The outlines of the head of the femur and acetabulum were irregular and there was irregular condensation of the bones adjacent to the joint space. The right acetabulum was protuberant. Hypertrophic osteo-arthritic changes were noted in the knee joints.

The initial electrocardiograms exhibited regular sinus rhythm and left deviation of the electrical axis. The pattern of left ventricular preponderance appeared in 1948.

The urine exhibited all of the physical and chemical properties due to the presence of homogentisic acid, as described in the body of the paper. The patient was not anemic and the total and differential white blood cell counts were normal. The blood Wassermann test was negative. There was no disturbance in the blood level of glucose, urea nitrogen, creatinine, uric acid, total protein, albumin, globulin, alkaline and acid phosphatase, total cholesterol and cholesterol esters, chloride and icteric index. The galactose tolerance test was normal.

For the most part the patient was comfortable. She spent most of each day in a wheel chair. Pains in her shoulder and knee joints were relieved by aspirin tablets. In April, 1944, she suffered from left otitis media and in July, 1945, she developed an abscess in the left breast which was incised and drained. On three occasions in April, 1948, she complained of epigastric and precordial distress which was associated with paroxysmal auricular tachycardia lasting approximately one hour. An aortic diastolic murmur was heard for the first time in April, 1948. In July, 1948, more persistent supraventricular tachycardia developed along with fluid at the base of the left lung. Following digitalization these did not recur. She remained relatively well until early in the morning of May 1, 1949, when she was found dead in bed, having been in good spirits when seen an hour previously.

On postmortem examination the calvarium had a deep yellow-brown color. The inner aspect of the dura mater contained patches of black pigmentation. The cut surfaces of the cerebral arteries were stained black. The structures of

the neck appeared normal except for black pigment in the thyroid, tracheal and laryngeal cartilages and in the cut surfaces of the large arteries. The costal cartilages, costochondral junctions and cartilages of the sternoclavicular joints were blackened and there was gray discoloration of the ribs. The lungs appeared normal except for black discoloration of the bronchial cartilages. The heart was enlarged to the left because of left ventricular hypertrophy. There was black pigmentation in the commissures of the pulmonary valve. The aortic valve appeared mottled black. The annulus of the aortic valve and of the mitral valve was calcified. The coronary arteries contained numerous atheromatous placques which were stained black. The endocardium and myocardium of the left ventricle contained many fibrotic areas. Black pigmentation was present at the insertion of the chordae tendinae in the papillary muscles. The inner surface of the aorta had a mottled black appearance due to pigment deposits in atherosclerotic, calcified areas.

The vertebral bodies were markedly atrophic and appeared to be fused in the lumbar region. The intervertebral discs were black and protruded. The joints of the extremities were not examined.

There were numerous diverticuli of the sigmoid colon.

SUMMARY AND CONCLUSIONS

1. The salient aspects of the literature on alcaptonuria and ochronosis have been reviewed.

2. Case histories and postmortem findings of three patients suffering from alcaptonuria with ochronosis and arthritis have been presented. Metabolic studies were carried out in two of them.

3. The metabolic disturbance appeared to be of equal severity in both patients since the rate of homogentisic acid excretion varied approximately to the same degree in each with comparable variations in the protein content of the diet.

4. Both patients converted nearly all of intravenously administered L-phenylalanine to homogentisic acid. Their ability to convert D-phenylalanine to homogentisic acid was markedly limited and differed in degree. L-phenylalanine was catabolized to homogentisic acid more rapidly than D-phenylalanine. Nearly all of L-tyrosine fed by mouth to one patient was excreted as homogentisic acid.

5. The rate of excretion of homogentisic acid was not altered by large doses of nicotinic acid, thiamine chloride, riboflavin, pyridoxine, pantothenic acid, vitamin C, vitamin K_1 with and without sodium glycocholate, oleum percomorphum, viosterol, crude and purified liver extracts, insulin, adrenal cortical extract and tyrosinase. Ingestion of brewers' yeast caused an increase due to its high protein content.

6. The nature of the metabolic disturbance in alcaptonuria has been discussed in light of current knowledge of tyrosine and phenylalanine oxidation.

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Seminars on Gastrointestinal Physiology

Current Views on the Physiology of the Gastric Secretions*

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THE physiology of the digestive tract is still a young science. Consequently the worker who attempts to present a well rounded picture of this branch of physiology finds that his canvas contains more blank spaces than solid areas of comprehension. However, its growth is rapid, and so one would do well to stand off and examine the canvas in perspective at frequent intervals. For the clinician who seeks to interpret disorders of the stomach in the light of its physiology, such periodic review is likewise of great importance, particularly for his proper evaluation of medical and surgical procedures newly proposed for their management. The following discussion endeavours to present a picture of our current knowledge and hypotheses concerning the physiology of the mammalian stomach, and represents the background of ideas being used for these clinical purposes as well as for the design of new experimental ventures.

The essential functions of the stomach include (1) temporary storage of ingested food while it is being reduced to a semi-liquid state; (2) secretion of chemical substances required for such liquefaction; (3) modification of the chyme so as to be tolerated by the duodenum; and (4) the controlled ejection of this chyme into the duodenum. It is generally believed that salivary digestion of carbohydrates may continue in the stomach for twenty or thirty minutes following ingestion of food. The magnitude and duration of this process varies with the amount of gastric juice contained in the viscus at the beginning of the meal and the rapidity of its formation during the eating process. In any case the hydrolysis of carbohydrates in the stomach does not appear to be important for gastric physiology proper, and we may pass directly to such morphologic considerations as are pertinent to our objective.

STRUCTURE OF THE GASTRIC MUCOSA

The cellular sources of the gastric secretions are all in the mucosa. Major blood vessels are distributed in the outer layers of the stomach wall; the mucosal lining contains only vessels of smallest magnitude, and there is now good evidence for the existence of an extensive network of A-V shunts. Similarly, the major nerves to the stomach course only in its seromuscular layer. The cholinergic innervation derives from the two infradiaphragmatic trunks of the vagus, each of which contains fibers from both the right and left vagi, above the diaphragm. However, there is reason to believe that some of the vagal fibers are buried in the esophageal wall and enter the cardiac end of the stomach apart from the main trunks. A small fraction of the total cholinergic supply may derive also from the 4th and 5th dorsal nerve roots, at least in the dog, and penetrate the serosa along with the splanchnic nerves. Cholinergic and adrenergic nerves all lead to a three-dimensional meshwork which is usually described as consisting of three plexuses: subserous, myenteric (Auerbach's) and submucosal (Meissner's). This meshwork is extremely complex in regard to its origins and intercommunications, and little is understood about the ultimate distribution and functions of its terminal fibrils. About the glandular epithelium there is much more understanding, and it is already possible to draw a picture which correlates well with secretory function

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in spite of considerable gaps in our knowledge of both.

The entire mucosal surface—comprising the cardiac and pyloric regions as well as the corpus and fundus—is covered with tall columnar epithelium, overlaid with a layer of viscous mucus.

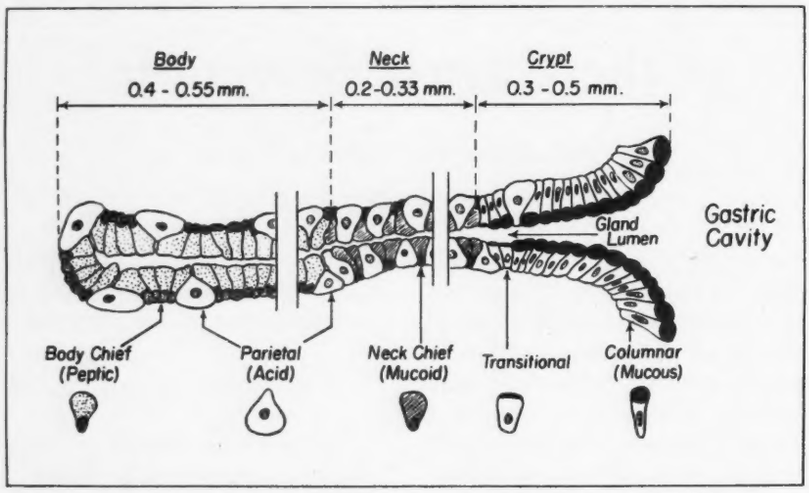


FIG. 1. Diagrammatic representation of gastric gland tubule (dimensions approximate).

Dotting this sheet of surface cells are huge numbers of minute openings (three million or more for adult man) which represent the mouths of the foveolas or crypts. Each one of the latter communicates with one or more (an average of 4.3 is sometimes cited for adult man) narrower collecting tubules, consisting of first the neck and then the longer and narrower body of the gland. The fundus of the latter rests on a basement membrane which is separated from the submucosa by a very thin muscle sheet, the muscularis mucosae. The folding of the mucosal layer into rugae, grossly evident under most circumstances, is a consequence of the greater area of mucosa than of muscularis propria as well as of muscular activity in the stomach, but it bears no significance for secretory function. In general, the surface epithelium extends into the crypt, undergoing a gradual transition from tall columnar cells at the mouth to the low variety near the neck of the gland. (Fig. 1.) At the junction between crypt and neck there occurs a smaller type of cell, designated the transitional cell, the function of which is generative rather than secretory; it is believed to give rise to the columnar cells on one hand and the tubular epithelium on the other.

In the corpus and fundus three varieties of epithelial cell occur, each with a specific kind of secretory activity: neck chief (also designated mucous neck or mucoid) cells, body chief (peptic or central) cells, and parietal (acid, oxyntic or border) cells. These are interspersed with other varieties, among them argentaffin and mast cells, and intestinal epithelium on occasion, although the normality of occurrence of the latter is still a moot point. Of the several secretory elements the parietal cells predominate in the neck, where they often border directly on the lumen of the gland instead of being literally "parietal," whereas the peptic cells predominate in the body of the gland.

In the area of mucosa adjacent to the gastroesophageal orifice, sometimes extending into the lowermost portion of the esophagus, the typical fundic glands undergo a gradual transition, with progressively vanishing density of parietal and peptic cells. These are designated the cardiac glands. In the distal end of the stomach, down to the pyloric ring, a region of tubular glands occur, short and wide, with numerous tortuous branches. Except in a narrow transitional zone these comprise only mucous cells, i.e., columnar epithelium typical of the corpus and another variety designated pyloric gland cells.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF GASTRIC SECRETION

It is almost impossible to collect pure gastric juice from the intact stomach of man or experimental animals because of contamination by

saliva and the secretions regurgitated from the duodenum. When obtained from isolated auxiliary stomachs or pouches, the material is usually fluid, transparent (except for occasional suspension or colloidal dispersion of insoluble mucin) and colorless. Its specific gravity is low, usually about 1.002. Its freezing point depression is generally about the same as that of plasma or somewhat greater. Variations in osmotic pressure of the blood, induced experimentally, are reflected promptly in the osmotic concentration of the gastric juice. Viscosity, adhesiveness and cohesiveness are about the same as for water, except in the near or complete absence of HCl, when these physical properties are strikingly high because the secretion is predominantly mucus. In the absence of blood such mucous specimens are also colorless but of widely variable opacity, depending on their content of suspended mucin and desquamated epithelium. This variety of secretion is also slightly hyperosmotic with respect to blood.

The chemical composition of gastric juice reflects the complexity of its composition, being a mixture of all the inorganic ions found in other body fluids, free HCl most of the time, bicarbonate ion when acid is absent and a number of organic constituents. Included among the latter are several enzymes and their precursors, three or more different mucins and their degradation products, the intrinsic factor of Castle, and a miscellany of other substances derived in minute amounts from interstitial fluid and cellular disintegration. Qualitatively, bicarbonate may or may not be present in anacid juice, for the addition of minute amounts of HCl to the non-acid secretions will convert their alkali bicarbonate to free CO_2 to an extent dependent on the proportion of the two varieties of fluid. However, specimens with pH above 7 probably always contain their full complement of alkali proteinate and phosphate, along with any unneutralized bicarbonate. Such mixtures of acid and alkaline secretions are said to have an *acid deficit*, a term which possesses much merit physiologically although it is now employed but rarely. In the pH range between 7 and 3.5 bicarbonate is invariably absent and the protein buffers of the alkaline secretions exist in varying degree as protein chloride or *combined acidity*. Only when the buffer salts of the non-acid fluids have been completely neutralized is HCl present as such, corresponding to *free acidity*. The pH value adopted as a boundary between free and combined acidities is 3.5, equivalent to a titrimetric acidity of about 1 mEq./L.* The *total acidity* represents the sum of free and combined acidities but it is evident from the foregoing that the secretion of minute amounts of gastric acid may never be recognized because of its conversion to, and disappearance as, carbonic acid. Phosphate is found only in small amounts; Cl ion occurs in relatively high concentration, regardless of the acidity, and derives from all the constituent secretions either as HCl or as alkali and alkaline earth chlorides. However, such *neutral chlorides* may be formed in part by interaction of bicarbonate and HCl. Of all the cations the concentration of Na in gastric juice remains at or below its level in other body fluids, whereas K may attain a concentration many times that in plasma; Ca and Mg are also present but their concentrations are never greater than in the blood. According to current concepts none of these cations derives from the same cellular source as the acid.

It appears from the foregoing that the chemical anatomy of pure gastric juice varies widely and according to no obvious pattern but this seeming chaos is gradually being reduced to simple design in terms of the several individual secretions which compose the mixed gastric fluid, each having a different cellular origin and a more or less well defined chemical anatomy of its own. In fact the inorganic composition of any mixture of these distinct secretions, in any particular proportion, can now be predicted with considerable confidence from their chemical interactions and their mutual degrees of dilution. Let us, therefore, proceed to a detailed consideration of each of the physiologic entities by itself.

ENZYME SECRETION

Pepsin. This enzyme, foremost among all those present in gastric juice, is synthesized by the body chief cells and extruded in the form of a zymogen, called pepsinogen. Nothing is known about the intracellular processes concerned with the synthesis of this proenzyme except that it is formed continuously and stored as granules which move toward the luminal end of the cell and disappear during the act of secretion.

* The physicochemical unit of 1 mEq./L. [or one millinormal (mN)] is numerically identical with the "clinical unit" or "degree of acidity," but the first is preferred because it offers a scientific rationale whereas the last two are both arbitrary.

Whether pepsinogen secretion is accompanied by water and electrolytes is still undetermined; if so, their contribution to the total volume of gastric juice is exceedingly small. In fact it may be that when the inactive enzyme is ejected by the body chief cell, it tends to remain in the lumen of the gland until it is washed into the cavity of the stomach by the fluid acid secretion.

Pepsinogen has been isolated in crystalline form. It is a protein of molecular weight about 42,000, inactivated reversibly both by boiling and by treatment with alkali at pH 9.0 or above. On addition of acid, however, the zymogen is converted to active pepsin by an autocatalytic reaction in the course of which a third substance with the character of a pepsin inhibitor is also liberated. The molecular weight of the active pepsin in crystalline form has been found to be about 38,000 whereas that of the inhibitor (also a nitrogenous substance) is of the order of 5,000.

The function of pepsin in the stomach is to hydrolyze dietary proteins to proteoses and peptones in preparation for their complete degradation to small polypeptides and amino acids in the small intestine. As with all the digestive enzymes, the rate of peptic hydrolysis varies with the pH of the reaction mixture, being nil at both high and low values and maximal in a restricted range between. Pepsin differs from all the others, however, in having a pH optimum of activity in the neighborhood of 2. Values usually cited vary from 1.2 to 2.4, depending on the substrate employed; this interval corresponds to a range of 74-4 mN in terms of titrimetric acidity. The hydrolytic potency of the enzyme vanishes below pH 0.7 (*ca.* 240 mN) on one hand and above pH 4.5 (less than 1 mN) on the other. Hence, for the purpose of evaluating antacids in terms of their chemical efficiencies, apart from their clinical potencies, it has been proposed to designate this higher pH of 4.5 the *proteolytic neutralization point* for pepsin in contrast to 3.5 which is the *free acidity end-point*.

Rennin. The coagulation of milk, involving proteolysis of soluble caseinogen to proteins of lower molecular weight, can be effected by pepsin in HCl but there is also a specific enzyme, rennin (pH activity optimum = 4–5) which catalyzes this hydrolysis. After years of controversy it is now generally accepted that rennin is found in the 4th stomach of the calf but is absent from human gastric secretion, infant as well as adult.

Other proteases have also been reported as occurring in gastric juice but their specific functions are not always evident. Chief among these is *cathepsin* (pH optimum = 4–5), a protease which is activated by cyanide and cysteine. The occurrence of this enzyme in the stomach wall is beyond question but the evidence for its occurrence as a major component of gastric secretion is indirect and still awaits confirmation. Gastric tissue contains a specific gelatinase, and the ability of gastric juice to liquefy gelatin can be ascribed to a small amount of this enzyme as well as to pepsin.

Non-proteolytic Enzymes. Urease and carbonic anhydrase both occur in considerable quantities in the tissue of the stomach but they are negligible factors in its secretion. *Carbonic anhydrase*, which catalyzes both the formation of H_2CO_3 from CO_2 and H_2O and its decomposition in the pH range 6–9, is found in the surface epithelium as well as the parietal cell. It is now known to play this important role during the formation of HCl (q.v.) but its function in the gastric mucous cells is still not evident. Urease (pH optimum = 6.5–7.5) is also an endoenzyme with no evident function in the stomach but it may be that it is involved in the formation of NH_3 from urea for the purpose of neutralizing the HCl. *Lysozyme* is a highly specific carbohydrate-splitting enzyme (pH optimum = 5.3) which occurs normally in both secretion and tissue, but only in small amounts. Its concentration in gastric juice of the ulcerated stomach may be elevated markedly but in the stomach tissue no increase is evident except in the area immediately adjacent to the lesion. Its presence under these conditions is a reflection of a localized defensive reaction, and in no way an etiologic factor. Finally, there is *lipase*, which can be extracted from both normal tissue and its secretion. That it is an exoenzyme of the stomach is now generally denied, and that portion of it which is not endoenzyme appears to be one of the constituents of the regurgitated duodenal fluid. The fact that lipase has a pH activity optimum at 4–5 also argues against its possessing any function in the gastric juice, the pH of which is usually about 2.

Exocrine-endocrine Partition of Pepsinogen. As with all other digestive enzymes pepsinogen is classified as *exocrine* because it is ejected from its parent cell into the lumen of a gland or viscus. However, a small fraction of the total zymogen is ejected by the cell also into the adjacent inter-

stitial fluid, from which it is transported ultimately to the urine. This fraction of the pepsinogen (designated *uropepsinogen*) is in effect an *endocrine* product of the cell, even though it serves no hormonal function. A similar distribution of total enzyme production between exocrine and endocrine fractions is already known to occur with other digestive enzymes (e.g., amylase from the salivary glands and the pancreas, and lipase from the latter) and it may well be a general phenomenon throughout the alimentary canal. In the case of pepsinogen the total quantity excreted through the kidneys in, say, three hours is roughly proportional to that secreted simultaneously into the stomach, at least while the latter is in a fasting state, and amounts to roughly 1 per cent of the total. This is true for man with gastric or duodenal ulcer as well as with no ulcer disease (individuals with the achylia of carcinoma or pernicious anemia put out no uropepsinogen) so that a fairly good measure of the rate of pepsin secretion in the stomach can be obtained without gastric intubation, merely by determining the simultaneous rate of uropepsinogen excretion. Data on blood levels of this proenzyme are not yet available because of the inadequacy of our present assay methods.

ACID SECRETION

It is a truly remarkable characteristic of the alimentary tract that at each of its levels electrolytes, adequate both in character and in quantity, are present to adjust the pH to a value suitable for the efficient operation of its digestive enzymes. Thus the enzymes of the salivary, intestinal and pancreatic glands require pH levels around 7, and so they are provided with sources of bicarbonate and phosphate buffers. In the stomach, on the other hand, pepsin acts best at pH values around 2, and so its glands contain a unique type of cell for the production of a strong inorganic acid (HCl) in quantities large enough to neutralize all the alkaline salts of the saliva and food, and then to reduce the pH of the food mass still further. In addition to this, gastric HCl possesses two other important functions: initiation of the autocatalytic conversion of pepsinogen to active pepsin; also, extensive sterilization of the gastric chyme by destroying bacteria, such as streptococci, staphylococci and Escherichia coli more or less completely.

In what cell does the stomach elaborate HCl at a concentration which would prove destructive in any other organ? Since pH levels of 3 or less can be detected in the minute canaliculi which communicate between the tubules of the gastric glands and the interior of the parietal cells, the latter are properly called "oxyntic." Furthermore, there is a good correlation between the density of these cells in microscopic volumes of the gastric mucosa and the content of acid determined microchemically in immediately adjacent regions. Also, the occurrence of carbonic anhydrase in the parietal cells, taken together with recently reported inhibition of acid secretion in Heidenhain pouches by a newly synthesized inhibitor of this enzyme, lends additional support to our acceptance of the parietal cell as the source of gastric acid. Acid pH's, however, have never been detected within the cytoplasm of the parietal cells, from which it must be inferred that the HCl is probably synthesized at or within the wall of the *intracellular canaliculus*. This minute structure is actually a tubular arborization within the cell and leads into the lumen of the gland, either directly or by way of an *intercellular canal* which passes between the zymogen cells. The intracellular canaliculi are almost never seen in tissue sections prepared from a resting stomach because they are collapsed when empty. During active secretion, however, they are amply distended and therefore readily discernible.

As for the intracellular chemistry of HCl formation, a sufficient number of facts are now available to provide an acceptable hypothesis:

(1) The chlorine is derived from the blood stream as inorganic chlorides (i.e., of Na, K, etc.) without being stored in significant amount.

(2) The time required for the passage of chloride from the vascular system to the lumen of the stomach may be as little as one minute, the salts being converted to HCl immediately prior to its secretion and without intermediate formation of organic chlorides.

(3) Since we are concerned with the secretion of an electrolyte, the character and concentration of its accompanying electrolytes are of paramount importance. Although pure parietal secretion, as it is ejected from the cell, has never been collected, it is generally believed to be a pure solution of HCl at a concentration in the neighborhood of 165 mN. It contains no metal cations whatever; for some time there was reason to think that K occupied a peculiar position in this regard, being present at a con-

stant concentration of 7–10 mN, but this has recently been shown not to be the case. Anions other than Cl^- are also absent; however, other members of the halogen family (i.e., Br^- and I^-) when injected into the blood stream also appear in the parietal secretion, as HBr and HI.

(4) An HCl solution with a concentration of approximately 165 mN is slightly hypertonic with respect to blood, and such hypertonicity of the parietal secretion is confirmed by freezing point depression measurements as high as 0.62°C. This small differential in osmotic concentration may possibly result from the synthesis of new solute and the utilization of some of the water in this synthesis.

(5) Simultaneous with the secretion of HCl the venous blood from the stomach undergoes an increase in its concentration of bicarbonate ion. Total chloride decreases very little, if any, presumably because this ion and water occur in the gastric juice in a ratio not radically different from that in the blood. The rise in bicarbonate content, however, reflects the production of alkali simultaneous with the production of HCl, so that in essence the parietal cell effects the hydrolysis of neutral chloride with the ejection of free HCl in one direction and of NaOH neutralized chiefly by H_2CO_3 in the other. Of all the organs the stomach alone presents this anomalous situation that, during its activity, its venous blood may be more alkaline than its arterial supply. These small changes in blood concentrations resulting from normal gastric activity are sometimes reflected in an increase in CO_2 tension of the alveolar air during the first half hour postprandially and in the "alkaline tide" of urine—although the latter can be explained also as a consequence of decreased respiratory activity associated with postprandial drowsiness, or of a preponderance of alkaline constituents of the meal itself. In clinical situations involving prolonged secretory activity with loss of HCl by vomiting or gastric lavage these changes in blood and urine become very marked, even to the extent of frank alkalosis.

(6) That the parietal cells contain carbonic anhydrase has been known for some time but evidence to establish its role in relation to HCl secretion was not available until recently. This enzyme possesses the peculiar function of speeding up the reaction represented by the equation

$$CO_2 \text{ (in solution)} + H_2O \rightleftarrows H_2CO_3$$

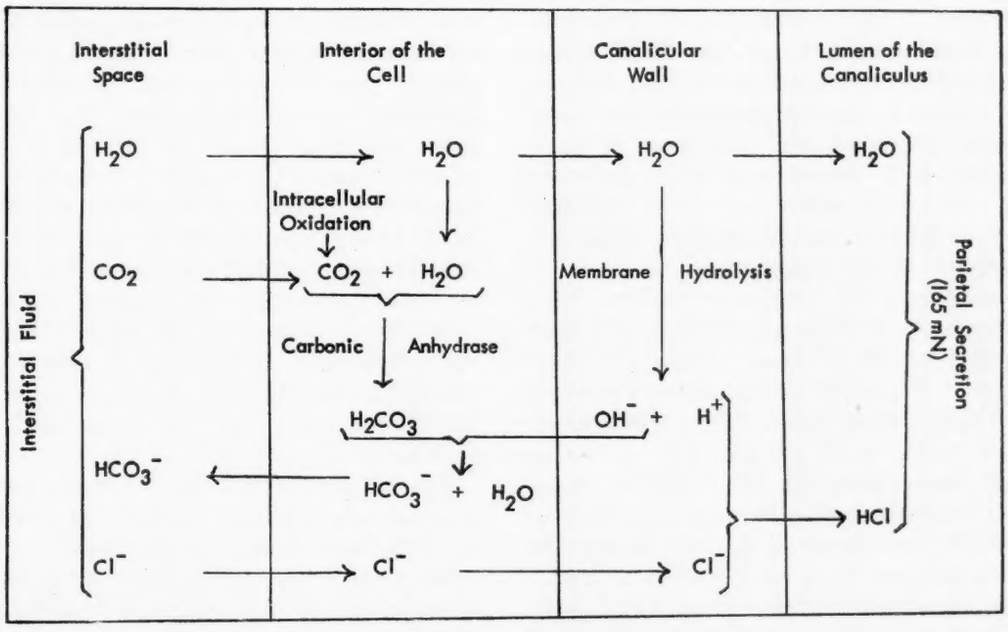
toward its equilibrium point, regardless of the direction. In its presence the rate of hydration of CO_2 *in vitro* may increase one thousand fold or more. Some recently synthesized sulfonamide derivatives are now available which are exceedingly effective inhibitors of carbonic anhydrase activity *in vitro*. Recent studies with one of these (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, called compound No. 6063) has proved it to be a potent, although not quite complete, inhibitor of the secretory response to histamine injections in living animals as well. Therefore, there is now little doubt that carbonic anhydrase plays an important role in the production of gastric HCl but whether the enzyme is essential to the process is still unsettled.

What hypothesis can be offered concerning the mechanism of HCl formation in the stomach which will unite these half dozen observations into a consistent and meaningful whole? The most comprehensive of several proposals advanced to date is the *membrane hydrolysis theory*, formulated chiefly on the basis of our own investigations during the past twenty-five years. Since the uncontaminated parietal secretion is entirely devoid of cations other than H ion, and contains no anion other than Cl ion, it is assumed that there exists within the cell a membrane that is permeable only to these two ions and water. This membrane constitutes the wall of the intracellular canaliculus—recently reported to be an organized structure with a thickness of the order of 0.5μ—which constitutes the boundary surface between the cytoplasm of the cell and its route of communication with the lumen of the gastric gland. Inorganic (neutral) chlorides, derived from the blood stream and interstitial fluid, enter the cell with water, carbon dioxide, oxygen and nutrient substances as these are required for secretory activity. On stimulation water moves out of the cell into the canaliculus taking with it H^+ and Cl^-, the only dissolved particles which can possibly move along with it. The concentration of the solute is 165 mN, because this concentration of HCl is isosmotic with the cytoplasm and slightly hyperosmotic with respect to the blood. Thus the reaction by which acid is formed from the alkali chlorides (BCl) may be envisaged as a hydrolysis which occurs within the specifically permeable wall of the intracellular canaliculus, i.e.,

$$BCl + H_2O \rightleftarrows HCl + BOH.$$

The base, BOH, must be neutralized immediately as it is formed or else the pH of the cyto-

plasm would increase inordinately and the cell would be destroyed. For this purpose the cell uses its buffers, phosphate, protein in small degree, perhaps even some lactic acid of intracellular metabolic origin, but chiefly carbonic acid. The latter is derived in part from the interstitial fluid and in part from intracellular oxidation. The chemistry of this hydrolysis and neutralization may be described in three apparently different ways, i.e., as two stepwise reactions; or as a double decomposition between H_2CO_3 and BCl with the formation of HCl and $BHCO_3$, the latter being excreted into the interstitial space in exchange for an equivalent amount of BCl; or simplest of all, an interchange of Cl^- and HCO_3^- between interstitial fluid and cytoplasm, and simultaneously, an electrically balanced movement of H^+ and Cl^- across the hydrolyzing membrane from cytoplasm to lumen of the canaliculus, but no movement of cation (B^+) whatever. These three formulations are only different ways of viewing the same chemical processes. According to the last of these views, we have the following detailed schema:



The slight hypertonicity of this system relative to the extracellular fluid generally results from an increase in concentration of solute particles caused by the generation of H ion and HCO_3 ion from molecular CO_2, and also by the utilization of some of the water in this reaction. The substitution of HCO_3^- for Cl^- in the interstitial fluid accounts for the increase in alkaline reserve of the blood, the associated change in alveolar CO_2 tension, the alkaline tide in the urine, and thus the alkalosis which accompanies marked loss of gastric juice by vomiting or lavage. Hydrochloric acid formation can proceed in the absence of carbonic anhydrase because of the small uncatalyzed portion of the reaction by which H_2CO_3 is formed, but in the presence of the enzyme the rate is increased considerably. Hence if activity of the enzyme be blocked by compound No. 6063, HCl production will be reduced drastically but not entirely. Other buffer systems of the parietal cytoplasm may also continue their contribution to the chemical dynamics of HCl production during carbonic anhydrase inhibition but the extent of their contribution is not yet clear.

A number of colored substances, when injected parenterally, are poured out by the mucosa into the lumen of the stomach, simultaneous with acid production, and for this reason it is claimed that they are actually secreted by the parietal cells on stimulation. Chief among these pigments is neutral red, which is often used under special circumstances as an indicator of the ability of these cells to produce HCl. The peculiar amphoteric characteristics of these compounds lend plausibility to this claim but they also can be used to explain their transport through the interstitial spaces of the mucosa rather than through the cells

themselves. In either case this phenomenon as yet contributes nothing to our understanding of the intimate mechanism of HCl production.

MUCOUS SECRETIONS

Mucus is the secretory product of the columnar cells. This secretion contains one or more complex organic compounds, called mucins, and probably all the inorganic ions of other body fluids. As ejected by its parent cell its pH is about 7.4, but as it is collected from a gastric pouch or the intact stomach the pH may be less than this (because of contamination with minute amounts of parietal HCl) or as high as 9.0 (because of loss of CO_2 on exposure to air). The ability of pure mucus to neutralize acids is variable and may be equivalent to concentrations as high as 80 mN. Specimens of this anacid secretion may be entirely acellular or may contain huge quantities of columnar epithelium and leukocytes, depending on the type of stimulus. Mucus is characterized chiefly by its high viscosity but its cohesiveness and its adhesiveness to cell surfaces and food particles are also important physical characteristics. Frequently, however, it occurs in moderately fluid forms as well as highly viscous ones. This may be explained by the fact that the viscous secretion undergoes spontaneous liquefaction at 37°C. but there is also the possibility that these more fluid specimens are, wholly or in part, the product of a second variety of secretory element—the neck chief cell—and therefore properly designated *mucoid secretion*. However, this possibility is still hypothetical.

Several chemically different mucins have been recognized, without any indication of their cellular sources. All of them are complex combinations of a protein of unknown character and a mucopolysaccharide. The carbohydrate moiety contains acetylglucosamine, galactose and sometimes glucuronic acid; glucose, mannose and fucose may also be present though in lesser proportions. Some of the mucins contain H_2SO_4 (in different ratios); one without it, i.e., the neutral polysaccharide, is identical with blood group A specific substance. Specimens of acid gastric juice contain degraded forms of the mucins in solution as well as undissolved. The former have been fractionated by precipitation with acetone and trichloracetic acid into "mucoprotein" and "mucoproteose," but what relation exists among the native mucins and these degradation products is still obscure.

The functions of the mucous secretions in the stomach are all protective. The viscous material, forming a layer on the inner surface of the stomach, protects it against mechanical, thermal and chemical trauma, for any form of irritant process evokes an immediate thickening of this surface layer. If the irritant be particularly destructive to the superficial columnar cells these are promptly desquamated and their highly viscous contents re-enforce the surface layer. Replacement of these shed cells by migration and regeneration occurs with such speed that the entire process of cellular desquamation and replacement must be considered to constitute an integral part of the protective mechanism of the stomach against autodigestion, peptic ulceration and possibly even cancer production (by endogenous carcinogenic agents). For these reasons the concept of a protective gastric mucous barrier which is made up of two components is coming to play an increasingly important role in our thinking. According to this hypothesis the layer of mucus constitutes the first line of defense and the deeper layer of columnar cells the second. In addition to the lubrication afforded by the layer of mucus it serves to adsorb pepsin, to neutralize HCl and to wash away traumatic substances which impinge upon it, being replaced immediately following such washing away. All of these functions contribute to making mucus a most effective protective agent. Laboratory and commercial extracts of "mucin," administered orally, have been found to be inadequate as substitutes for the protective mucous barrier; this failure is readily understandable since these preparations possess neither the adhesiveness or the cohesiveness of the natural mucus, nor the ability to withstand the disintegrating action of HCl, nor can they replenish themselves following such disintegration.

For purposes of analysis of the physicochemical relations of mixed gastric juice, particularly the relations among the concentrations of its various ions, the term *alkaline component* is frequently used in conjunction with *acid component*. The latter comprises parietal secretion alone; the former is a mixture of variable composition which includes all forms of mucous secretion, pepsinogen secretion, cytoplasm of desquamated cells and transudate of interstitial fluid which probably occurs in the lumen of the stomach also. Another hypothetic fluid, designated the *dilution secretion*, is also encompassed by the

alkaline component but this may prove to be identical with either the mucoid secretion or the transudate. Thus the term alkaline component does not represent a real physiologic entity but is merely a convenient expression which will vanish as we learn more about the individual secretions which it includes.

STIMULATING MECHANISMS

In the same way that the chemical makeup of mixed gastric juice has definitive physiologic meaning only in terms of the individual secretions which compose it, so the character of the total secretory response to a meal acquires meaning only in terms of several categories of stimulation, i.e., neural, hormonal, secretagogue and mechanical. Analysis according to these categories is also important for understanding the responses to a great variety of chemical substances which are employed for clinical testing of gastric secretory function and for therapeutic purposes. The following discussion treats these several categories of stimulation not only in terms of their routes but also in terms of the specificity of their end-organ responses.

Neural Stimulation (*Psychic, Cephalic or Vagal Phase of Secretion*). This may arise peripherally or centrally as part of a long reflex chain. The efferent routes of communication with the gastric glands are along the vagi in most cases, the sympathetic nerves exercising a restricted effect which is not clearly understood. It is slowly becoming evident, also, that some cholinergic nerves with secretory function may reach the stomach wall along with the sympathetic supply from the spinal cord. The complexity of the vagal pathways, especially in man, and the numerous structural variants which occur among different individuals, contribute greatly to the current deficiency of knowledge in this area. Afferent impulses to the vagus center arise from receptors of vision, taste and smell. The mere thought of savory food or its hypnotic suggestion also serves as a stimulus to secretion, and conditioned reflexes can easily be developed on the basis of such gastronomic experiences. Thus mastication of food—without its entering into the stomach, i.e., *sham feeding* accompanied by expectoration, by regurgitation from an esophagus that is completely obstructed at the cardiac orifice or by loss through an esophageal fistula—induces gastric secretion by a combination of these several receptor mechanisms, all operating via the medulla and vagal trunks. On the other hand the ingestion of inert substances like oatmeal gruel exercises little or no influence on these reflexes. Transection of the vagi, if complete, makes all stimuli within this category ineffective. The latent period for neural stimulation is short relative to that for other modes of stimulation, only a few minutes at the most, but the response may persist for two or more hours when the primary stimulus is of a high order of palatability for the subject, or if its "emotional" content be high in other respects.

The secretory end organs for vagal stimulation are multiple. It activates both the acid- and the enzyme-forming cells; in fact, a high enzyme activity generally serves to identify a specimen as vagal or vagomimetic in origin. Electrical and pharmacologic stimulation of the vagus trunks evokes the output of viscous mucus also, but only if the intensity of stimulation is low. Otherwise, the secretory product is typically fluid, with high acidity and pepsin activity. However, although neural fibrils from Meissner's (submucosal) plexus are known to pass to the parietal and zymogen cells, their communication with the mucous cells has never been demonstrated unequivocally. Furthermore, vagal stimulation induces muscular activity of the stomach, and this in turn squeezes mucus out of the folds of the mucosa and the crypts so that the output of mucus following such stimulation is evoked indirectly in some degree, and maybe entirely.

Secretory responses to sympathetic stimulation also merit consideration in this context. Observations of an increased output of acid and pepsin are scant and not at all consistent, but electrical stimulation of freshly cut splanchnics in anesthetized animals may give a steady secretion of typical viscous mucus, at moderate rate, for a prolonged period. In contrast to mucus obtained by parasympathetic stimulation, the pepsin content of this material is low. Also, the response to sympathetic stimulation is inhibited by ergotamine but not by atropine whereas parasympathetic stimuli are blocked characteristically by the latter.

Hormonal Stimulation (*Gastric or Gastrin Phase of Secretion*). This second type of stimulus is an endocrine substance called *gastrin*. This hormone is formed in the gastric wall, especially in the pyloric region, but by what cell is unknown. It can be transported to its effector organ by way of the circulation but its direct diffusion through

the interstitial fluid to the nearby gastric glands is also a possibility. Gastrin has already been extracted from gastric tissue in sufficiently pure state to be used in experimental animals. Contrary to early evidence the most recent preparations of this humoral agent are free of the vascular activity of histamine; but this does not preclude its being some unidentified derivative of this compound.

Stimulation of gastric secretion through the gastrin mechanism can be evoked in either of two ways: (1) mechanical distention of the pyloric antrum by liquids, solid food or even an inflated balloon; and (2) contact with the antral mucosa of chemical substances derived chiefly from meat and other proteins by partial digestion, (particularly proteoses, peptones and nitrogenous extractives). Although this is distinctly a humoral mechanism for stimulating gastric secretion and its latent period is relatively long, its initiation depends on a local reflex process. The receptors of this reflex lie in the gastric mucosa. This is evidenced by the observation that topically applied procaine can block the initiation of the hormone mechanism by chemical or mechanical means but not the action of a purified gastrin preparation which has been injected intravenously.

Gastrin, be it derived exogenously or endogenously, activates only the parietal cells. Pepsin is put out in very small quantity, probably only by being washed out by the acid fluid, and mucus practically not at all.

Exogenous Secretagogue Stimulation (*Intestinal Phase of Secretion*). Like the second mode of secretory stimulation this also is a humoral mechanism but it makes use of a great variety of chemical agents which are absorbed into the general circulation by the small bowel. These substances are all exogenous and their action does not involve a local reflex process in the intestinal wall, as evidenced by the failure of bowel distention or topically applied procaine to influence their ability to stimulate secretion in the stomach. Chief among these substances are nitrogenous meat extracts, liver extract, proteoses, peptones, a few of the amino acids, alcohol and agents like histamine and some of its derivatives. Carbohydrates and fats are ineffective, although soluble soaps are not. The mode of action of such substances has generally been thought to be by direct stimulation of the effector cells because such action was not affected by vagal section. However, there is mounting evidence that a neural process, not yet understood, may be involved in this third phase of secretion as well. This is particularly the case for the amino acids, which may act centrally in spite of the early findings. Also, it is now well established that the secretory response to these secretagogues can be blocked by systemic atropinization, and that the response to histamine, at least, can be augmented synergistically by vagal stimulation.

There seems to be only a single variety of effector cell for these secretagogues, the parietal cell. Gastric juice collected in response to this mode of stimulation has a low content of both pepsin and mucin, provided the vagal phase has been obliterated entirely. The volume of fluid secreted in the stomach in response to the intestinal phase appears to be relatively low, and considerable doubt is often expressed about its importance in the entire secretory organization of the stomach.

Mechanical Stimulation. Rubbing of the mucosa with a rough object, in contrast to distention, must also be considered a secretory stimulus. This process evokes the secretion of viscous mucus only. Acid secretion fails to occur, as is evident in a completely resting stomach pouch and, although pepsinogen is found mixed with the mucus, it seems likely that its presence results from a squeezing-out effect rather than true stimulation of the zymogen cells. Mucus extrusion under these circumstances may also be a matter of external pressure as well as of actual stimulation of the surface cells to eject their secretory product actively. Even if this be so, the response to mechanical irritation fits in with the protective character of this secretion, and rubbing must be considered a true mode of stimulation for the mucous epithelium.

Note on Resting Secretion. The current terminology of gastric secretory physiology includes a number of terms which imply a fourth phase of gastric secretion: *basal secretion, continuous secretion, interdigestive secretion, night secretion, appetite secretion.* These all lend themselves to interpretation in terms of the three stimulating mechanisms already described and are clearly the result of such stimulation, particularly the vagal. However, a possibility still remains that the parietal cells are capable of some degree of spontaneous activity in the absence of all these stimuli. Although the interdigestive phase of secretion in fasting patients is sometimes eliminated following complete surgical vagotomy for peptic ulcer, and this lessens the likelihood that such unstimulated activity does occur, this evidence

for its non-existence cannot yet be accepted as conclusive.

INHIBITING MECHANISMS

Neural. There is suggestive evidence that the vagi contain inhibitory as well as stimulatory fibers for gastric secretion. Nausea induced by foul-smelling odors, reflex distention of the bowel, painful stimuli from other regions of the body, certain anxiety states and hypnotic suggestions of various allied experiences, all are capable of causing a reduction in gastric acidity in man. However, definitive pathways for such effects have never been demonstrated and the problem presents a virgin field for investigation.

Hormonal (*Enterogastrone*). When fat or sugar is brought into contact with the duodenal mucosa, gastric secretion is inhibited markedly. Among the many endocrine substances generated by the intestinal mucosa there is one which is specific for this reaction. This substance, called enterogastrone, has already been isolated in sufficiently pure form to permit injection into man as well as experimental animals. A counterpart to this agent also occurs which inhibits motor activity in the stomach; this also is called enterogastrone but is apparently distinct from the secretory inhibitor. Marked sugar and fat content of the intestinal chyme are the two outstanding stimuli for evoking this hormonal process. Enterogastrone exerts its influence against the peptic as well as the acid cells, regardless of whether the secretion is induced initially by histamine or by vagal stimulation.

Urogastrone. A substance with essentially the same physiologic properties as enterogastrone has been isolated from urine of both males and females under a variety of conditions, and this has been designated urogastrone. It seems probable, however, that these two inhibitors do not have a common origin and that urinary output of the latter may be related to pituitary activity.

EFFECT OF MISCELLANEOUS CHEMICAL SUBSTANCES

In the foregoing analysis of stimulating and inhibiting mechanisms reference has been made to a few pertinent chemical agents but there are many others, pharmacologic and dietary, which are of special interest to the clinician. Many of these are used as stimuli for the testing of gastric secretory function, and clear understanding of their activity in terms of these mechanisms is important.

Histamine and Its Analogues. This compound and some of its derivatives recently synthesized (e.g., 3-beta-aminoethylpyrazole) are classified as secretagogues. Administration is parenteral, but oral ingestion is effective in high dosage. These are the most potent stimuli of gastric secretion known today, and it is suggested that histamine of endogenous origin may play a special role in relation to gastric secretion.

Ethyl Alcohol. When ingested in high concentrations (e.g., above 80 per cent), this substance acts chiefly or solely as a mucigogue-desquamating agent but in dilute solution it functions as a true secretagogue, giving juice of high acidity and low pepsin content. These effects become manifest whether ethyl alcohol enters the blood stream directly or by absorption in the stomach, intestine or rectum. There is some evidence that alcohol operates only indirectly, by release of histamine in the lungs, but this awaits confirmation.

Pilocarpine, choline, acetylcholine and mecholyl all act as vagomimetic agents. They stimulate the gastric glands to produce acid, pepsin and mucus even when the mucosa has been deprived of its vagal innervation, and are presumed to act at the residual neuroglandular junction.

Insulin falls in a different category from the vagomimetic agents. It is also effective following intravenous injection but only, as a rule, if the blood sugar level is reduced to 50 mg./100 ml. or less. This results in central stimulation of the vagus and a typical neural phase response of the gastric glands, which can be prevented by the simultaneous intravenous injection of glucose, complete vagal transection or atropinization (at least in part).

Certain amino acids (e.g., β-alanine) and *barbituric acid derivatives* (e.g., 3, 3-dimethylallyl barbituric acid), synthesized during recent years, have been reported as being direct vagocentric stimuli, without intermediation of a hypoglycemic state. Confirmation of such activity has been difficult, however, particularly for those new compounds which have a high degree of toxicity.

Caffein administered parenterally or orally is a stimulus to gastric secretion in man and cats but the dosages required are relatively higher than for histamine. In dogs a response is not elicited at all unless the dosage be of the order of 2,500 mg. Classification of its mode of action is impossible as yet. Since the response is enhanced synergistically by histamine or alcohol

and is diminished by vagotomy, it may be that the drug affects gastric secretion by way of its general stimulating effect on the central nervous system.

Atropine and other anti-cholinergic blocking agents exercise an inhibitory action against vagal and vagomimetic stimuli, as already described. Atropine also interferes with the secretory response to histamine and to alcohol but interpretation of this awaits clarification of the synergistic relation between these secretagogues and vagal influences.

Banthine is another anticholinergic drug which reduces the response of parietal and zymogen cells to neural stimulation. It differs from atropine, however, in possessing a dual site of action: (1) at the neuroglandular junctions, and (2) at the ganglia of the parasympathetic (and also the sympathetic) nervous systems. Its potency as an inhibitor, relative to that of atropine, is still a moot question, largely because of a quantitative difference in side reactions which permits a one hundred fifty-fold difference in dosage by oral administration.

Inorganic acids, especially when administered parenterally, tend to reduce gastric secretory function in response to any of the usual stimuli—drastically, if the stimulus be vagal; relatively little, if it be secretagogue. In fact, any experimental situation which tends to lower the CO_2 tension of the blood, even without a change in its pH, exerts this inhibitory effect. It may well be that such acid-base influences on gastric secretion operate not as pharmacologic agents in the usual sense but in relation to the supply of CO_2 which they afford to the parietal cell for its production of HCl in conjunction with carbonic anhydrase.

Alkalies, as might be expected from the foregoing, tend to stimulate secretion. This has given rise to the concept of an *acid rebound* which is said to occur some hours following the ingestion of $NaHCO_3$, after its intragastric neutralizing action has already been effected and its influence on the acid-base balance of the body generally has become manifest.

Meat extractives, *liver concentrates* and the like all are powerful secretagogues, as has already been discussed, and for this reason were commonly used as stimuli before histamine became commercially available.

Fats, in general, exercise an inhibitory effect by way of the enterogastrone mechanism. However, in the late stages of their action, after they have been digested and saponified, they exert a stimulating action—perhaps as secretagogues, although this has not been clearly established. Fatty acids administered directly also exercise such a stimulating action.

Carbohydrates as a rule manifest no stimulating or inhibiting action on the stomach.

Oatmeal gruel merits special mention because of its classic use as a testmeal or stimulus for clinical testing of secretory function. Its freedom from pleasant odor and taste, especially when prepared without salt, excludes its acting by way of the vagal mechanism, and the speed of its action suggests that its influence as a contributor of secretagogues by way of the intestinal phase is probably not very pronounced. This leaves only the hormonal mechanism, and it is most likely that gruel operates by way of gastric distention and consequent evoking of the gastrin mechanism.

Condiments generally act as stimuli but this occurs indirectly by way of the taste apparatus and therefore involves only the neural mechanism. The essential oils, such as oil of cloves and of peppermint, when administered topically in sufficient amounts act as mucigogue-desquamating agents but not as stimuli to acid secretion.

Coffee, *defatted cocoa* and so forth are stimuli in part by way of the gustatory-vagal reflex, in part by way of their caffein content.

CONCLUSION

The foregoing presents a unified picture of the most important physiologic processes involved in gastric secretion. Limitation of space has necessitated omission of some items of clinical interest, such as absorption in the stomach, the ranges of variation in gastric acidity associated with different disease states and the causes of these group differences, and the various systemic effects of total gastrectomy. In spite of the omission of these items and of much detail about those topics which have been discussed, this picture has already demonstrated its value as a background for the physiologic interpretation of major gastric disorders and many of the test procedures and therapeutic measures employed in their management. An attempt has been made to differentiate confirmed from unconfirmed observations, and both of these from hypotheses. These differentiations in scientific methodology are highly important and the clinician no less than the investigator should keep them in mind constantly.

Quantitative Tests of Gastrointestinal Function*

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RELIABLE methods for the quantitative measurement of gastrointestinal function are needed to measure the degree of impairment which characterizes or accompanies disorders of the gastrointestinal tract. At present we possess technics for the analysis of normal and disordered gastrointestinal function which are rudimentary when compared, for example, with the highly refined methods available for investigation of renal and cardiopulmonary function. The variety of processes in the gastrointestinal tract makes precision difficult but it must be admitted that this does not alone explain the paucity of dependable methods and measurements.

It is the purpose of this review to summarize such quantitative tests as are now in use, to define their value and to indicate the need for further development. The present review deals only with the gastrointestinal tract proper and the pancreas. The liver and biliary system do not fall within its scope.

For the purpose of this analysis the complex and synchronized processes of digestion will be considered schematically in three functional groups: (1) secretory, (2) transport and (3) absorptive. The tests to be evaluated will be considered in these terms. It should be clearly recognized that function tests have only limited significance. They furnish little evidence of structural alteration, are of limited diagnostic value and have little etiologic significance.

I. SECRETORY TESTS

Tests of Gastric Secretory Function

The study of the secretory function of the gastric mucous membrane has centered almost exclusively upon the secretory products obtained directly by aspiration of the stomach. The difficulties of obtaining the pure products of gastric secretion by gastric tube aspiration are apparent. For one thing, there is contamination of the gastric juice by swallowed saliva and regurgitated material from the small intestine (succus entericus, bile and pancreatic juice). The introduction of test meals into the stomach leads to further contamination. Moreover, quantitative study of gastric secretion is difficult because of loss of juice by gastric emptying. However, these defects can be minimized. A dental saliva collector and careful instruction of subjects will reduce salivary contamination. Aspiration of intestinal contents along with gastric juice through a double-lumen tube will reduce intestinal regurgitation.[1] The dye dilution indicator technic with test meals offers a method for estimating the proportion of secretion and test meal present in each sample of gastric contents[2] although there are inherent difficulties in this method, too. Finally, continuous aspiration by mechanical suction, or very frequent intermittent suction, will reduce the amount of juice lost by gastric emptying.

Tests of Parietal Cell Function. Most gastric tests depend upon the ability of the parietal cell to secrete hydrochloric acid. Since the formation of acid has been presented in this series in considerable detail, few pertinent points need be recapitulated. HCl is secreted as a solution in concentration of approximately 165 mEq./L., nearly isosmotic with blood. Since "pure" gastric juice is an admixture of this parietal juice with the non-parietal or alkaline component of mucous secretion, the concentration of HCl in a specimen of gastric juice is a function of the relative proportions of the two components.

Archaic terminology and methods have persisted in regard to the measurement of HCl. Acidity is often reported in "clinical units" or "degrees." Since this number is equivalent to the milli-normal concentration (mN) or milli-

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equivalents per L., and the latter designation is almost universally used in regard to the concentration of solutes in other body fluids, there is no reason why the outmoded terms should be retained.

The conventional indicators for determining free and total acidity are Toepffer's reagent (dimethylaminoazobenzine), which has it characteristic color change in the pH range 2.8–3.5, and phenolpthalein, which has its characteristic change in the range pH 8–10. Considerable improvement in rationale has been made by Hollander's suggestion that pH 3.5 and 7.0 should be used for determining free and total acid, respectively; the former corresponding to a solution with a free acidity of 1 mN, and the latter to physiochemical neutrality. For this purpose bromphenol blue and phenol red are quite satisfactory. When these are used as buffered standards with a micro-burette, specimens as small as 0.5 ml. can be titrated with an accuracy of ± 2 mN. Although the pH of the gastric juice can easily be determined with the glass electrode (and pH information is of value in relation to enzyme activity curves), the reliability of acid concentration when determined titrometrically is considerably greater than when recalculated from a pH determination. When color contamination prevents a colorimetric titration, electrometric titration can be substituted.

Direct measurement of parietal cell function: In measuring parietal cell function the following questions are of physiologic and clinical interest: (1) Can a given patient secrete HCl? (2) What is the concentration and amount of HCl secreted during the physiologically significant phases of gastric secretion? (3) What is the response of a given patient to a standard secretory stimulus? (4) What is the maximum secretory capacity of the stomach of a given individual?

Ability of the parietal cell to secrete HCl: The simplest test of parietal cell function, i.e., whether a given stomach can secrete any HCl at all, has some clinical importance. The diagnosis of Addisonian pernicious anemia is incompatible with secretion of HCl by the gastric mucosa. No authenticated cases of duodenal ulcer have been recorded without acid. Gastric, anastomotic and jejunal ulcer occur only extremely rarely in the presence of achlorhydria.

Histamine test: To test this function, the parietal cell should be stimulated by a substance acting directly upon the cell and not upon an intermediate pathway. Histamine (β-imidazolylethylamine), which can stimulate the isolated gastric mucosa *in vitro* and the stomach in the absence of its extrinsic nerve supply, is the drug of choice at present. Because of its side effects, predominately vascular, the upper limit of dosage is sharply limited; but 0.01 mg. of histamine base per kg. of body weight given subcutaneously is an adequate and tolerated dose. Since histamine base, histamine dihydrochloride and histamine disphosphate have a molecular weight of 111, 184 and 307, respectively, the dosage of the commercially available products must be adjusted accordingly. Recently 3-beta-aminoethyl pyrazole, an isomeric analog of histamine, has been used for testing parietal cell function,[3] since it stimulates acid formation with few side effects. Although enough clinical studies are not available for assessing this compound the early reports are encouraging.

In performance of the histamine test the position of the tube should be checked fluoroscopically and the response followed for at least one and one-half to two hours. It may be necessary to repeat the test several times to detect the secretion of small amounts of acid.

In interpreting the histamine test one should also consider the significance of the "combined" acid. Thus if a small amount of acid is secreted in the presence of a large amount of mucus, no free acid but only combined acid may be found. The pH of this mixed fluid may also be helpful. Since mucus is secreted at a pH very close to that of blood and interstitial fluid, pH levels below 7 would indicate that some acid parietal secretion has been added to the mixture.

In critical situations the failure of the mucosa to respond to histamine should be checked with other stimuli. Ihre[4] has shown that insulin hypoglycemia occasionally will be more effective. Watkinson and Jones[5] have convincingly demonstrated that it may be necessary to perform gastric aspiration for many hours, and to use food as a stimulus for acid secretion in some individuals with gastric ulceration.

Neutral Red Test. Neutral red appears in the juice of an actively secreting stomach following its parenteral administration and is believed by many investigators to be excreted by the parietal cell. Recent evidence indicates that this parallels the secretion of acid and is dependent on both the rate of acid secretion and the concentration of dye in the blood.[6] Accordingly, a large num-

ber of clinical observers have stressed its use as a test of parietal cell function, especially in achlorhydria. But several factors limit the value of this test. (1) Neutral red is cleared by the liver, appears in bile and can be regurgitated into the stomach, thus vitiating the test in some instances. (2) The appearance in gastric juice of a wide variety of basic dyes, (i.e., dyes whose cations are chromophoric), is not a proof of secretion since distribution of these dyes may be by passive diffusion depending on their pK's.[7] (3) Neutral red can be excreted in animals even when acid formation has been suppressed by distention.[8] (4) The transport of neutral red from the blood through the parietal cell is a most indirect measure of whether the cell can secrete HCl.

Tests of the phases of gastric secretion: A variety of gastric secretory tests have been devised in order to measure the concentration and amount of parietal secretion during the physiologically different phases of gastric secretion. The purpose of these is not only to set up standards for the range of normal values but also to determine whether derangements of specific phases of secretion occur in clinical disorders.

The interdigestive or basal secretion is that secretion which occurs in the absence of all intentional and avoidable stimulation.[9] Why the human stomach continues to secrete in the absence of overt stimulation is not known, but phasic vagal impulses are probably involved in the process. It is difficult to determine when a patient is in the basal gastric state but conditions similar to those for measuring the basal metabolic rate are usually employed. Bloomfield and his co-workers have stressed this measurement as "the simplest and most useful procedure in the study of clinical gastric physiology."[10] The interdigestive secretion has been studied for varying periods of time, day and night, in normal subjects, and in patients with duodenal and gastric ulcer.[4,10–14] Lack of information regarding the reproducibility of such tests reduces the value of most reports. It is of interest that although there are marked differences in the absolute values obtained by different investigators, all observers agree that under standardized conditions the secretory rate in patients with duodenal ulcer is elevated above that of normal individuals or of those with gastric ulcer.

Because of the difficulty of controlling night secretion in duodenal ulcer[15,16] emphasis has been placed recently on the nocturnal phase of interdigestive secretion. It is doubtful whether measurement of basal secretion at night gives much more valuable information than tests performed during the day.[13] More studies of both aspects in the same patients are needed before the point can be settled.

The digestive phases of secretion are conventionally divided into three groups depending on the site of origin of the stimuli causing secretion. While tests of *the cephalic phase*, elicited by sham feeding, are an attractive clinical possibility, the fragmentary published material on this phase in man excludes this at present. *The intestinal phase* is probably of minor significance for normal digestion and no tests of this phase have been developed. The most widely used clinical test of *the gastric phase* of parietal secretion is the Rehfuss test which measures the response to a carbohydrate meal (gruel, toast and tea, or arrowroot cookies and tea). Samples of gastric contents are aspirated at ten- to fifteen-minute intervals for one or two hours, and the concentration of free and total acid is determined as well as the amount of food residue. This crude test is usually justified as a rough method for estimating the work of the stomach. Since it is a weak stimulant of the parietal cell, this test cannot be used to answer the question whether the parietal cell can secrete any acid at all. Also, the introduction of a large volume of fluid dilutes the gastric juice secreted. The addition of phenol red as an indicator of dilution permits estimation of that fraction of the aspirated material due to test substance and that portion secreted. In actual experimental analysis significant errors arise from the absorption of water from hypotonic test meals under certain circumstances.[17] The only feature which recommends the Rehfuss fractional analysis at present is the large number performed.[18]

Serial test meal: An attempt to meet the limitations of the conventional fractional analysis has been made by J. N. Hunt[19] who used a non-digestible citrus pectin meal with a dilution indicator and aspiration of gastric contents at fixed intervals on successive days. After demonstrating that the pattern of gastric emptying of this meal was exponential, he was able to calculate the amount of acid secreted during the test meal. This test has not been applied to clinical disorders and awaits evidence that gastric emptying in these states also follows some linear pattern.

Response of the parietal cell to a standard parenteral stimulation: Histamine: Because of the limitations of the test meal technic the response of the human stomach to parenteral stimuli has been studied extensively. It is disappointing, therefore, to observe that although histamine has been used for many years there are few good quantitative data on the response to this drug. The data of Ihre[20] and Levin et al.[13] for one-hour continuous aspiration of the stomach following fixed doses of histamine are among the satisfactory studies. Unfortunately, Pollard's extensive data of 988 cases record only the maximum ten-minute period of secretion.[21] All observers, despite differences in absolute values, are in agreement that duodenal ulcer patients secrete more acid than do normal or gastric ulcer patients. Diagnostic use of this procedure is limited by lack of information about reproducibility of results from day to day. Ihre, who performed two to five tests on thirteen subjects, noted variations up to 25 per cent in regard to volume of juice secreted.

Insulin: Insulin-induced hypoglycemia is a feasible parenteral stimulus but entails discomfort for the subject as well as frequent blood sugar determinations. The carefully collected data of Ihre[4] and Chinn et al.[22] are the only available information at present.

Maximum secretory capacity: The side effects of histamine prevent the determination of maximum secretory capacity, a measurement which could be used in determining the functional secretory reserve of the stomach. An approach toward this problem has recently been made by W. I. Card and his co-workers[23] who have attempted to measure "secretory cell mass" by extrapolating from histamine dose-response curves. Their unpublished studies suggest that differences in acid output in different stomachs may be related mainly to differences in parietal secretory cell mass. Obviously important if confirmed, this type of analysis is a step in the right direction.

Indirect measurement of parietal cell function: The attempt has been made to measure the secretion of HCl indirectly without intubation by using an exchange quininium cation resin which interacts in the stomach with HCl to form quinine hydrochloride, some of which is absorbed and excreted in the urine.[24] Interpretation of this test depends on the detection of the quinine in the first and second hour urine collection periods. In most cases the decision as to presence of HCl is easily made but in individuals with small amounts of the compound in the second hour specimen of urine quantitative measurement is needed. Other cations (Na, K, Ca, Mg) present in gastric and intestinal juice may react as well as H^+. Since the method measures only free HCl and is not a quantitative measure, it seems most useful as a screening method for mass use.

Summary of parietal cell tests: (1) Histamine is the drug of choice to determine whether an individual can secrete any HCl but it may be necessary in critical situations to use neural (insulin) and humoral (food) types of stimuli, as well as to lengthen the period of observation beyond the conventional one-hour study. (2) Quantitative measurement of basal secretion is the simplest test of level of functional activity presently available. Much more evidence regarding reproducibility in large groups of individuals is needed. It should be noted that in all tests investigated, the rate of secretion of HCl is markedly elevated in the duodenal ulcer subject above the normal or the gastric ulcer subject. This is usually ascribed to vagal hyperfunction but without full proof.

Tests of Gastric Enzyme Function. Peptic cell tests: Of the several gastric enzymes only pepsin has been studied intensively. The adaptation of the pepsin method of Anson and Mirsky to gastric juice[25] and the use of commercially available lyophilized bovine hemoglobin for the substrate[26] furnish a satisfactory method for determining pepsin activity.

Most clinical studies have dealt only with the concentration of pepsin in juice obtained in the fasting state[27] or following histamine[28] and no significant differences have been found by most observers between normal subjects and those with ulceration of the stomach and duodenum. Only in the large series of Vanzant and her co-workers,[29] who studied pepsin concentration following an arrowroot cookie meal, were there significant differences between normal subjects and duodenal ulcer patient; but in this study there were individuals without ulcer who had markedly elevated concentrations.

A more significant measurement is the determination of the rate of secretion (output per unit time) of pepsin. Histamine is not a suitable test since it is a doubtful stimulant for pepsin. The studies of Ihre[4] indicate that insulin can be used for this purpose but requires several blood sugar determinations as well as vagus nerve

continuity. Measurement of basal secretion of pepsin, in the same way that basal secretion of acid has been studied, recommends itself as a simple method for determining peptic cell activity. The studies of basal secretion of pepsin output by Ihre[4] (during twenty-minute periods), Janowitz and Hollander[14] (during three-hour periods) and Chinn et al.[22] (during twelve-hour nocturnal tests) all indicate significant elevation above the normal in duodenal ulcer patients despite some overlap in range of values.

Urinary pepsinogen: Convincing evidence indicates that the peptic cell secretes pepsinogen both into the lumen of the stomach and into the blood stream, from whence it is excreted in the urine. During basal secretion there is a direct relationship between the two fractions, approximately 99 per cent of the pepsinogen going into the stomach, 1 per cent into the blood and finally into the urine.[30] Urinary pepsinogen can thus be used as a measure of gastric peptic secretory activity and two groups of investigators have furnished evidence of its excretion in normals and in a variety of clinical conditions, especially peptic ulcer. I. A. Mirsky and coworkers[31] studied a group of twenty-seven healthy males in whom the range of values for the log hourly rate of uropepsin excretion (units/hr.) during overnight urine collection was from 0.00 to 0.97, with a mean of all values at 0.48. In thirty males with benign gastric and duodenal ulcers the "night" urine log hourly rate of excretion varied from 0.26 to 1.55, with a mean of 0.76 (twice that of the normal). Nine pernicious anemia patients had no urinary pepsin. In fourteen miscellaneous patients, who included five with functional complaints and five with gastric carcinoma, the values were in the normal range. In Janowitz, Levy and Hollander's series[32] of forty-four normals of both sexes without diseases of the upper gastrointestinal tract, studied during three-hour basal periods, the range of hourly rate of uropepsin excretion in units/hr. was 0–136, with a mean of 47 (SD mean ± 6). Thirty-five patients with duodenal or stomal ulcers excreted 114–373 units/hr., with a mean of 180 (± 12). Seven pernicious anemia patients excreted no pepsinogen, as did one patient with a total gastrectomy. Five patients with gastric ulcer and seven with gastric neoplasm gave results within the normal range of values. In both series urinary pepsinogen excretion was clearly elevated in active duodenal ulcer. While this approach has been exploited only for a comparatively short time, it offers a supplement to conventional technics. In patients with gastrointestinal bleeding or those with anastomotic gastrointestinal operations (where obtaining gastric secretion free of regurgitated intestinal contents is a practical impossibility), the indirect approach possesses advantages over gastric aspiration.

At present the basal rate of pepsin secretion is a satisfactory direct way of measuring the level of peptic cell activity. Urinary pepsinogen determination offers a supplementary approach. Both require further confirmation and extension. Other enzymes present in the gastric secretions do not at present furnish the basis for any quantitative tests of gastric secretory activity.

Tests Based upon the Non-Parietal or Alkaline Component. The alkaline component as a whole: The non-parietal or alkaline component includes the mucous secretion of the stomach with its inorganic and polysaccharide constituents which dilute and neutralize the acid component. This secretion has been measured by means of an acid reduction or neutralization test in which the ability of the stomach to reduce the acidity of instilled HCl solution is determined by periodic sampling similar to the conventional fractional test meal. This crude technic has revealed marked differences between duodenal ulcer subjects and normal controls. Following Hollander's analysis of the relation of acidity, neutral and total chlorides in acid gastric juice of pouch dogs,[33] J. N. Hunt[19] developed a method for estimating the amounts of non-parietal and parietal components in a sample of gastric juice. His evidence indicates that the non-parietal component considered as a whole, as well as the parietal component evoked by insulin and histamine, are elevated above normal levels in peptic ulcer patients.[34] This characterization of the non-parietal inorganic components as a whole is a fresh approach but the underlying assumptions require confirmation.

Organic components of gastric juice: Glass and his co-workers[35] have measured the organic components of gastric juice in terms of their tyrosine content after differential precipitation. On this basis they have divided soluble mucus into two constituents: a mucoproteose derived from the surface mucous columnar cells, and a mucoprotein ascribed to the mucoid neck cells. They have reported the range of values for these components in basal secretion, and following hista-

mine and insulin, in normals and in a variety of clinical categories. Clear-cut elevations of mucoprotein above normal were shown in subjects with duodenal ulcer, consistent with the increased secretion of all other components in these patients. Methods are urgently needed for measuring the carbohydrate moiety as well as the protein (tyrosine) content of mucus.

Insulin Test of Vagal Continuity. Determination of vagal intactness or interruption is of practical importance at present because of the continuing use of vagotomy in connection with many operations on the stomach for ulcer disease. Insulin induced hypoglycemia as the stimulus, and the measurement of free hydrochloric acid as the gastric response (as formulated by Jemerin, Weinstein and Hollander[36]) is the most practical and widely used test of vagal continuity. It is necessary to determine blood sugars during the test to be sure that values of 50 mg./100 ml. or less have been reached, and the ability of the mucosa to secrete HCl must be known in advance. A rise in concentration of free HCl of 10 mN or more has been arbitrarily set as defining a "positive" test, one which indicates continuity of the vagus. In the presence of anastomotic operations the use of free HCl as the end point is complicated by the presence of considerable "combined" acidity due to regurgitated intestinal juice.

Intestinal and Colonic Secretion

Quantitative data regarding the succus entericus, especially the secretion of Brünner's glands of the first portion of the duodenum, would be useful for investigating possible deficiencies in these secretions in peptic ulcer but no quantitative measure of these secretions has yet been presented.

The only measure of colonic secretory function used at present is the estimation of the fecal content of the mucolytic enzyme, lysozyme. This enzyme, which lyses several strains of saprophytic bacteria including Micrococcus lysodeikticus and which hydrolyses a mucopolysaccharide extracted from this organism, is present throughout the entire intestinal tract and its secretions. Meyer and his associates[37] recently discovered that the fecal concentration of lysozyme and its twenty-four-hour excretion is enormously elevated in active ulcerative colitis. The lysozyme titer in this disease may exceed the normal range (0.1–1 unit/G wet stool) by a hundredfold. Convincing evidence indicates that these large amounts of enzyme are secreted locally by the diseased colon. However, the meaning of this altered lysozyme secretion in colitis is not yet altogether clear. The original hypothesis that lysozyme is an etiologic agent in colitis is supported by the evidence that the enzyme can damage the colonic mucosa under suitable experimental conditions.[38] In conflict with this hypothesis are the observations that a variety of infected and granulating human and experimental wounds, both in and outside the intestine, have a high lysozyme content; that lysozyme does not liquefy colonic mucus; that no substrate for the enzyme has been found in the human colon; and that the lysozyme content of the colon can be correlated with the inflammatory reaction and granulation tissue present.[39] Lysozyme excretion in colitis may possibly represent a reaction to injury.

The viscosimetric method for the enzyme assay is reliable and fairly rapid but requires a mucopolysaccharide substrate which is difficult to prepare. Since the excretion of lysozyme has been shown to parallel the clinical activity of the underlying ulcerative colitis,[39] serial determination of lysozyme titers (twenty-four-hour excretion) may be useful in following the course of this disease.

Tests of Pancreatic Secretory Function

Secretin Test. Direct measurement of the external pancreatic secretion has been markedly advanced by the Lagerlöf double lumen tube which separately aspirates the gastric contents and the duodenal contents up to the ligament of Treitz.[40] The admixture of hepatic and biliary secretions in the second portion of the duodenum remains a problem but this is minimized when there is good reservoir function of the gallbladder. The use of this type of collecting tube and stimulation of secretion by the hormone secretin is the method of choice for pancreatic secretory study. This test has not been widely used because of limited supplies of commercially available secretin preparations. Recently two varieties in potent form with minimal side effects have become available in this country. There is some question, however, as to their relative freedom from the enzyme-stimulating hormone, pancreozymin. This may explain some of the discrepancies in amylase output which have been reported by different observers. It should be remembered that secretin is a stimulus for secretion of water and bicarbon-

ate ions by the pancreas, whereas its action on pancreatic enzymes is a "washing out" rather than a true stimulating effect.

All workers have used Lagerlöf's original dose of 1 clinical unit per kilo body weight (16 cat units) and have collected the pancreatic response for sixty to eighty minutes, usually 80; a dose which evokes a submaximal response in most individuals. Dreiling and Hollander[41] have summarized the range and means of values in normal subjects for volume output, maximum HCO_3 concentration and amylase output in the four largest series of published reports.[40-43] There is surprisingly good agreement for volume and bicarbonate responses—less satisfactory agreement for enzyme output. These authors' values, especially their minima, furnish satisfactory standards for evaluating the secretin response in clinical disorders.

The response to secretin is a function of secretory pancreatic mass and patency of the duct system. The concentration of bicarbonate ions varies with the rate of secretion so that its concentration can be expected to parallel the volume rate.

In acute pancreatis transient abnormalities in secretin response occur, but at times when it is difficult to perform the test. In the small number of patients studied during the first two weeks of the illness some depression in the volume response and amylase output has been noted. Serial determinations during and following the acute phase may help to indicate whether these patients will go on to the chronic form of the disease or whether the acute phase has been an exacerbation of underlying chronic disease.

In *chronic pancreatitis* most observers have found a depression in the secretin response, especially in the volume-rate and the bicarbonate concentration, with some diminution in enzyme output. However, good data for correlating the histologic appearance of the gland with its secretory output do not exist.

In the only series of cases of chronic pancreatitis in which the secretin test was compared with fecal analysis for nitrogen and fat (see below), the secretin test disclosed evidence of insufficiency of external secretion more often than did the fecal studies.[44,45]

The alterations in response in pancreatic *carcinoma* depend on the extent of replacement of acinar tissue by tumor, the location of the tumor and the degree of obstruction of ducts. Carcinoma of the tail of the pancreas does not alter the secretin response. Carcinoma of the head and body usually leads to reduction of the volume response with little change in the concentration of the contents of the juice but the published cases are few.

Biliary pigment response to secretin: Purified secretin stimulates the formation of bile by the liver, increasing its volume rate mainly by water secretion.[46] The entrance of this augmented flow of bile into the duodenum interferes with quantitative collection of pancreatic juice but does furnish evidence regarding gallbladder reservoir function.[47] Serial determination of the bilirubin concentration or the icterus index of successive duodenal specimens has been used as a test of gallbladder function and correlates well with radiographic study of the gallbladder. Several types of pigment concentration curves are known. One type occurs in the presence of a normal gallbladder which receives and concentrates bile, so that the bilirubin content of some duodenal specimens falls to zero levels. In a second type there is a more or less continuous flow of bile into the duodenum because of a nonfunctioning gallbladder. In the third type no bile enters the duodenum because of complete biliary obstruction. A fourth type, indicative of incomplete biliary obstruction, has been described by Dreiling and Hollander[41] and is intermediate between the first and second types.

The pancreatic response to secretin and the pigment curve may help in the differential diagnosis of some cases of jaundice. Most patients with hepatitis have normal external pancreatic secretion.[41,48] In the presence of jaundice an abnormal pancreatic response to secretin tends to favor lesions of the head of the pancreas. A normal pancreatic response with an abnormal biliary pigment curve tends to favor lesions in the biliary tree. The secretin test is not helpful in differentiating between intra- and extrahepatic biliary obstruction.[49] Considerably more experience is necessary in order to evaluate this aspect of the secretin test.

Indirect Tests of Pancreatic Secretion. Some as yet quantitatively undetermined portion of the pancreatic enzymes finds its way into the blood and ultimately into the urine. This "exocrine-endocrine" partition, similar to that described above for gastric pepsinogen, furnishes an indirect approach to the appraisal of pancreatic function.[50]

Serum concentration of enzymes of pancreatic origin: Amylase has been the most frequently determined

serum enzyme of pancreatic origin. The determination is now usually performed by some variant of the Somogyi method[51] which measures the amount of reducing sugars released from starch in terms of glucose equivalents. The normal values range from 80 to 125 mg./100 ml. of serum. Amylase determinations have the obvious limitation that serum amylase is derived from several extrapancreatic sources (salivary glands and liver) and it is consequently difficult to interpret the quantitative significance of these serum levels.

Marked but transient elevations of serum amylase occur regularly in the course of acute pancreatitis. No correlation can be made between the degree of acute pancreatitis and this rise. It should be recalled that (1) rises may occur during the course of epidemic parotitis; (2) amylase can be absorbed from the peritoneum following rupture of the stomach or duodenum; (3) morphine and codeine may elevate serum amylase by inducing spasm of the sphincters of the pancreatic ducts; (4) elevated serum amylase values may result from impaired ability of the kidney to excrete the enzyme.[52] Although low values of the serum enzyme might be expected in destructive lesions of the pancreas, determinations of amylase are usually not helpful in these conditions.

Serum lipase: There are present in the serum at least two distinct esterolytic enzymes. One, an esterase, present also in liver and kidney, preferentially hydrolyzes esters of short chain fatty acids. The second, lipase, derived almost exclusively from the pancreas, hydrolyzes long chain fatty acids. At present the method of Cherry and Crandall[53] is used most commonly to measure serum lipase. The fatty acids released when 1 ml. of serum is incubated with an olive oil emulsion for twenty-four hours are titrated with N/20 sodium hydroxide. In normal individuals 1–1.5 ml. of alkali are required for this titration. In acute pancreatitis the rise in serum lipase may occur later than the amylase rise, but it is reported to persist longer. In carcinoma of the pancreas or in carcinoma of the ampulla of Vater lipase may be elevated more often than amylase, but this elevation depends on the degree of duct obstruction. With the replacement of functioning acinar tissue by neoplasm the serum value may return to normal or low levels.[54]

Perhaps a more recently described colorimetric method[55] which uses beta-napthyl laurate as the substrate may increase the clinical value of lipase determinations.

Proteolytic enzymes: There are no methods for measuring blood trypsinogen and chymotrypsinogen in the presence of the proteolytic inhibitors in the blood. It has been shown that these inhibitors are specific and are elevated in a variety of disease processes associated with cellular destruction.[56]

Following their experimental observation that intravenously administered trypsin resulted in a secondary and prolonged rise in the antithrombin titer of the plasma, Innerfield, Angrist and Benjamin[57] have noted similar marked elevations in antithrombin levels in patients with acute pancreatitis throughout the active phase of the disease. Their studies merit attempts at confirmation.

Serum enzymes following pancreatic stimulation: The possibility of investigating pancreatic function in terms of changes in blood enzyme activity following pancreatic stimulation has a good theoretic rationale. Clinically, however, the usefulness of this approach has not yet been securely demonstrated. For such a serum test of pancreatic function two clearly differential stimuli must be found: (1) In one instance the normal individual should respond with a reproducible rise in serum enzyme concentration and those with destructive lesions of the pancreas should fail to respond; and (2) a stimulus should be employed which elicits no rise in serum concentration of enzymes in normal individuals but does elicit a rise in those whose duct systems have been obstructed by pathologic processes.

To meet the first postulated case secretin, or secretin plus mecholyl, have been used in combination with morphine to produce sphincter spasm.[58] To meet the second case secretin alone has been used and there is some indication that this is a promising approach.[59] In both instances, however, the lack of uniformity in response or failure of response has proven a major obstacle. It may well be that a more effective combination of stimuli will be found. The development of pancreozymin in a form suitable for administration to man will probably advance this approach considerably.

II. TRANSPORT TESTS

The methods for recording gastrointestinal intraluminal pressures and motor activity measure forces involved in the transport of material through the gastrointestinal tract but

do not furnish direct evidence of transport. To measure the rate of gastric emptying and the rate of movement of chyme through the small intestine primitive methods are still used. They depend either on mechanical markers or dyes which are given along with food or on the movement of barium through the gastrointestinal tract as recorded by roentgen examination with films or fluoroscopy. The time of appearance of the marking substances in the stool is only partly a function of their passage through the stomach and small bowel; it is also a function of the time of habitual bowel movement. In the second instance adequate standardization of the barium meal has usually not been practiced and this approach assumes that some relationship exists between transport of food and transport of barium.

Gastric Emptying. Aspiration of the fasting gastric residum as usually performed furnishes only a very crude measure of the efficiency of gastric emptying since the volume recovered is a resultant of the rate of secretion and the rate of emptying. Testing the aspirated gastric contents during a gruel fractional test meal with iodine for starch furnishes misleading evidence in regard to emptying time. Radiographic observation of the stomach after a barium meal is merely a qualitative measure of the amount of ingested barium present in the stomach. The attempt to quantitate the pattern of gastric emptying by J. N. Hunt with his serial test meals has already been described.

Intestinal Transport. The rate of movement of a barium meal furnishes the only current device for quantitative study of intestinal transit time. The variation in reported values reflects lack of standardization. To correct this Lönnerblad[60] recently studied gastrointestinal transit time with a barium meal of fixed composition, adjusting the volume of meal to the subject's age. In 114 adults (eighteen to twenty-five years) receiving 200 ml. of meal the mean transit time was 178 minutes (range 38–549). In a recent unpublished study of twenty-four adults (ages sixteen to fifty-six) transit time of a 360 ml. barium meal ranged between two to six hours (mean of 3.4 hours). These findings in normal individuals indicate the wide variations encountered.

Insoluble carmine (0.6 G) is often given with a regular meal to measure the time required for the meal to traverse the entire tract. When we recently compared this method with the intestinal transit time of barium, the carmine marker time in twenty-four normal subjects on a regular diet ranged from ten to forty hours (mean 32 hours). The results showed no correlation with the barium time.

Evaluation of the significance of altered small bowel transit time awaits the development of a more physiologic "tagged" meal but it is problematic whether refinements in method will have corresponding clinical value.

III. TESTS OF INTESTINAL ABSORPTION

Two groups of tests are currently employed to measure intestinal absorption: (1) balance studies and (2) tolerance tests. In the balance studies the amounts of the major components of the diet ingested and excreted are measured over periods of several days to determine the net balance. This is time-consuming, costly and requires a high degree of patient cooperation. The tolerance tests measure changes in the peripheral blood concentration of specific components of the diet following standard loading of the gastrointestinal tract. These changes are indices of absorption, do not furnish absolute values of the quantities absorbed, and are influenced by mechanisms which remove them from the blood as well as absorb them. The two types of studies furnish complementary evidence. Practically, the tolerance tests find wider clinical application.

Balance Study of Intestinal Absorption

The balance method cannot be used to study carbohydrate absorption since an unknown portion of ingested sugars may fail to be absorbed but may be destroyed by bacterial fermentation in the intestinal tract.

Protein balance is measured in terms of nitrogen content of the stool which is in part nitrogenous products of the diet that have failed to be absorbed, in part bacterial flora nitrogen, in part nitrogen secreted into the gut. Fecal nitrogen does not vary directly with the dietary protein but is related to the weight of the dry matter of the stool, which is composed in large part of bacterial flora. In the normal adult on an ordinary diet fecal nitrogen usually amounts to 1 to 2 gm. daily. For acurate protein balance studies fecal nitrogen must be measured and the nitrogen of the diet should be determined. Although diarrhea *per se* does not markedly affect nitrogen excretion there may be a considerable loss of protein in the stool in patients with in-

flammatory diseases of the colon, in the form of exudate and blood. Increased fecal excretion of nitrogen in diffuse pancreatic disease has been reported by the majority of workers who have performed balance studies, values from 1.3 to 16.8 Gm. of daily fecal nitrogen having been found.[45] In a recent careful study of subjects with advanced pancreatitis[45] the range of values for daily fecal nitrogen varied from 1.7 to 5.6 Gm. (mean 3.2).

This feature has been stressed by Thayssen[61] as differentiating pancreatic disease from the sprue syndrome but more recent studies suggest that the range of values for fecal nitrogen in sprue may be similar to that seen in external pancreatic insufficiency.[62]

Fat Balance Studies. Fecal fat, measured as all ether-soluble substances extractable from the stool, represents less than 10 per cent of the ingested fat on ordinary mixed diets. As in the case of nitrogen, this fecal fat does not simply represent unabsorbed dietary fat but is in part secreted fat. With marked increase in dietary fat intake there is a considerable increase in the amount of fecal fat.

The partition of fecal fat into neutral fat and fatty acids has some qualitative significance since exclusion of pancreatic lipase from the intestine leads to an increase in neutral fat. This is not an absolute criterion for distinguishing pancreatic steatorrhea from other types, since some hydrolysis of fat can occur even if the pancreas is destroyed or its ducts occluded. In celiac disease, tropical and non-tropical sprue, and in some cases of diffuse ileojejunitis, free fat as well as fatty acids may appear in large amounts.

Tolerance Tests of Absorption

Carbohydrate Tolerance Tests. The *oral glucose* tolerance test, (determination of the serial concentration of blood glucose after the ingestion of either 1 gm. of glucose/kilo body weight, or a standard dose of 100 gm.) furnishes evidence of carbohydrate absorption, although the rate of gastric emptying, the previous diet of the patient and the mechanisms for removing glucose from the blood markedly influence the shape of the blood glucose curve. Curves of blood sugar concentration which show less than a 40 mg. per cent rise are usually considered as "flat" and interpreted as evidence of defective absorption. In celiac disease, sprue and some cases of diffuse regional enteritis, flat oral glucose curves are regularly found, although intravenous administration of glucose usually gives the expected rise. In patients with subtotal-gastrectomy the blood glucose curve may have an early peak followed by a sharp drop to hypoglycemic levels.[63]

An *oral galactose* tolerance test has been used by Althausen[64] who followed the blood concentration of galactose after an oral dose of 40 gm. in 400 ml. of water. In normal individuals the range of maximum concentration was 13 to 31 mg. per cent (mean 19). Diminished values have been found following intestinal resection, as well as abnormally high values (above 40 mg. per cent) in hyperthyroidism and Paget's disease; the last finding has not been generally confirmed.

Xylose tolerance test: The pentose, D-xylose, has also been used as a test substance in measuring absorption of monosaccharides, since D-xylose may be absorbed through a process of phosphorylation, and is excreted by the kidney where it is reabsorbed only to a very limited extent. Twenty-five gm. are administered in water and the five-hour urine output collected.[65] Normal individuals excrete between 4 and 6 gm. in this time, and sprue patients (only a small number have been studied) excrete between 1 and 2.5 gm.[66] Considerably more experience with both the galactose tolerance test and the xylose excretion test is needed before evaluation of their place can be made.

Protein tolerance tests: Several tests have been devised to measure the ability of the intestine to absorb individual amino acids. Most of these, however, have had only a very limited clinical trial. The blood concentration of alpha-amino nitrogen has been measured following an oral test dose of *glycine* (1–1.7 gm/kilo) in normal subjects and in infants with celiac disease and cystic fibrosis of the pancreas,[67] and in patients with massive resection of the small bowel.[68] Following a dose of 1 gm. of DL methionine/15 kilo body weight, the blood concentration of L-methionine has been studied by a microbiologic method[69] in a few clinical disorders involving diminished absorption. A gelatin tolerance test has been proposed which depends on both the pancreatic digestion of *gelatin* and the subsequent absorption of released amino acids. When 1.25 to 1.75 gm. of gelatin/kilo are fed, normal infants are reported to have rises of 6 mg. per cent of blood amino nitrogen, while infants with cystic fibrosis of the pancreas have "flat" curves.[67]

Lipid Tolerance Tests. Butter fat tests: Based upon the method of Nissen[70] in which total serum lipids are measured at intervals after the administration of 1 gm. of butter fat (given as heavy cream) per kilo body weight, Adlersberg and co-workers[71] have reported that four hours following the ingestion of 1.2 cc. of heavy cream (40 per cent fat content)/lb. body weight, sprue patients did not manifest the normally expected rise. Althausen[68] and his co-workers have used a butter tolerance test based upon turbidimetric determinations of the serum followed for periods of six hours after the oral administration of 60 gm. of butter. This has been applied to a few patients with massive resection of the intestine.

Vitamin A tolerance tests: Since vitamin A is fat soluble, it has been extensively used as a test substance following the discovery that its absorption is impaired in celiac disease. This has been extended to the study of cystic fibrosis of the pancreas and the sprue syndrome. At present the vitamin A tolerance test is a standard technic for investigating fat absorption.

As routinely performed either 7,500 I.U. of vitamin A per kilo (Althausen[68]) or 3 cc. of percomorph oil, 180,000 I.U., (Adlersberg[71]) are given by mouth, and the fasting and 4 hour plasma levels of vitamin A determined. Sprue patients regularly display flat curves with only a slight tendency for delayed rise. The use of water-miscible preparations gives higher values in normals but does not affect the general shape of the curve.[72] Although its clinical use is firmly established, the role of tissue stores and depletion in removing vitamin A from the blood is not yet understood. The flat curves in severe hepatic disease may represent impaired reserves and/or interference with intestinal absorption.[73]

Chylomicrographs: Following a fatty meal there is an increase in the number of bright particles of the peripheral serum seen with dark-field illumination. The curve of these chylomicrons following a standard fat meal has been used as a measure of fat absorption. As performed by Frazer and Stewart[74] peak counts of 150 particles per microscopic field are found in normal individuals, with variations depending on body build. Flattened curves have been reported in sprue by these workers.

IV. CONCLUSIONS

The preceding catalogue of current gastrointestinal tests reinforces the original statement of their limited nature.

Histamine can be used to determine whether a subject can secrete any hydrochloric acid. The level of parietal and peptic cell activity may be estimated from their output of acid and pepsin under basal secretory conditions. The determination of urinary pepsinogen offers a supplementary indirect approach to peptic cell function. A start has been made toward measuring the alkaline components of gastric juice. The insulin test can be used to determine the continuity of the gastric vagal innervation.

The excretion of lyzozyme parallels the clinical course of chronic ulcerative colitis.

The external secretion of the pancreas can be measured with some accuracy by means of the secretin test. The determination of pancreatic amylase and lipase activity in the blood is useful during the course of acute pancreatitis. Indirect measurement of pancreatic enzyme secretion by means of blood enzyme activity following pancreatic stimulation has not yet been satisfactorily established. The biliary pigment response to secretin is a fairly reliable measure of gallbladder function.

Only exceedingly primitive methods are available for the study of intestinal transport.

We possess an over-all method of considerable precision for studying intestinal absorption in the balance technic but this is not applicable to carbohydrates. Tolerance tests afford some index of absorption of the major components of the diet.

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Conference on Therapy

Treatment of Obesity

THESE are stenographic reports of conferences by the members of the Department of Pharmacology and of Medicine of Cornell University Medical College and New York Hospital, with collaboration of other departments and institutions. The questions and discussion involve participation by members of the staff of the college and hospital, students and visitors. A selected group of these conferences is published in an annual volume, Cornell Conferences on Therapy, by the Macmillan Company.

DR. GEORGE READER: Today we discuss the treatment of obesity, a problem faced by many of us every day. Dr. Robert Melchionna will open the discussion.

DR. ROBERT MELCHIONNA: The physician accepts the treatment of obesity with an apology, for there is no entity in medicine in which the burden of treatment is more completely reverted to the patient. The success of treatment will depend to a large extent upon how well the patient understands his problem and upon how he accepts his responsibility.

The importance of obesity as a contributory cause of serious systemic disorders is well recognized. While the average layman considers excess weight undesirable for esthetic reasons, the doctor sees it as a predisposing factor in arteriosclerosis, heart disease, hypertension, certain other peripheral vascular diseases, metabolic disturbances such as diabetes, gallbladder disease and many other illnesses. The significance of obesity is perhaps best seen in some of the life insurance company statistics, which clearly show that overweight is a serious hazard to health and life itself. These figures indicate that the optimal weight is 5 to 14 per cent less than the average weight.The so-called "average weights" are misleading in their implications. Many overweight people were included in the derivation of such averages, so that they cannot be taken as the optimal or normal values. A person normally will gain weight until the age of twenty-five or thirty. Thereafter the weight should decline slowly, until at sixty years the weight should be about 10 per cent less than that at thirty. Actually the reverse usually occurs, the weight climbing steadily through the middle years as fat is accumulated.

For the sake of completeness, rather than for discussion, the usual classification of obesity may be mentioned. First, exogenous obesity which is attributed to overeating and insufficient exercise; and second, endogenous obesity which is due to some inherent abnormality inducing a drop in the caloric consumption of the body. Often mentioned in this latter group are constitutional or hereditary factors, hypothyroidism, hypogonadism, adrenal cortical tumors, diabetes mellitus, hyperinsulinism, tumors of the pineal body, hypertrophy of the thymus, Froehlich's syndrome (dystrophia adiposo-genitalis), Laurence-Moon-Biedl syndrome, Cushing's syndrome, Dercum's disease, diseases of the brain and meninges, and lipodystrophia progressiva. As can be seen, practically all the endocrine glands are represented in this listing. Abnormalities of the endocrine system which affect adipose tissue are more intimately related to the distribution of body fat rather than to excessive accumulation of it. Actually, few obese individuals fall into the true endocrinopathic group, perhaps less than 3 per cent of the total. This is contrary to general opinion which blames all excess fat on "glands."

A detailed history, thorough physical examination and some laboratory investigation must be performed in every case of obesity to determine the presence of any of these endogenous factors or indeed of any of the diseases which often accompany obesity. These studies are not of primary concern in the actual therapy of obesity. Whether an endocrinopathy is present or not, overweight is fundamentally due to a disproportion between the caloric intake and the caloric consumption. No individual can escape the laws of energy conservation. But the physician must search for other diseases which will require specific treatment. In a patient with hypothyroidism with obesity, for example, the tribulations of losing weight will be eased, and of course the patient's general health improved, by simultaneous attention to the thyroid disorder.

Now in regard to treatment, I should like to consider simple psychotherapy, diet, medications and exercise. Proper attention is not ordinarily paid to the psychologic factors in obesity. It is important to know why the patient came for treatment, whether it was for esthetic reasons, from fear or because of some bodily symptom. These points will affect the strategy of treatment. Then one must determine the psychologic problems influencing the appetite. They are often deep-seated and not easy to uncover. The normal appetite adjusts the intake of food so accurately that it just meets, but does not exceed, the requirements of energy expenditure. When an emotional disturbance arises, this adjustment may lose its delicacy and obesity develops. The patient may be dissatisfied with his occupation and find distraction at his meal hour, during which he gorges himself with food and perhaps two or three cocktails. Or the patient may be a middle-aged woman whose children have married and are off by themselves and who now perhaps is lonely and finds her husband inattentive. She may turn to eating as a source of distraction and pleasure.

Indulgence in food often brings a respite from emotional problems and a large proportion of obese patients have some definite emotional problem. This must be helped if a lasting result is to be obtained. It is usually not necessary to refer these patients to a psychiatrist if the internist adopts an understanding, sympathetic and helpful attitude. On occasion, however, the psychosomatic factors are deep-seated and intangible. The patient develops an extreme pathologic craving for food which simulates that of the chronic alcoholic for drink.

A variety of reduction diets has been proposed but none possesses any unique virtue. The dietetic indication is simply to decrease the total fuel intake without destroying the proper balance of the ration or depriving the body of vitamins and minerals. The diet should contain a liberal daily allowance of protein, a minimum of 1 gm. per kg. of the estimated normal weight. Proteins are readily utilized, they have the greatest specific dynamic action and are said to stimulate thyroid activity. Concentrated carbohydrates, such as sugars and breadstuffs, and fats must be restricted. Diets, therefore, should exclude or minimize the use of rice, bread, potato, macaroni, pies, cakes, sweet desserts, free sugar, candy, cream, etc. They should consist of moderate amounts of meat, fish, fowl, eggs, cheese, coarse grains and skimmed milk. Generous amounts of fresh fruits, fruit juices, unsweetened stewed fruits and raw and cooked green vegetables can be allowed. Diets of this type contain adequate amounts of minerals and of water-soluble vitamins. Supplementary vitamins, particularly the fat-soluble vitamins including A and D, should be given to insure the patient against any deficiency.

There is no rule as to the total number of calories prescribed in the diet. Most authorities recommend that the total calories be arranged to induce a weight loss of 1½ to 2½ pounds per week and suggest diets varying from 300 to 1,600 calories. This, of course, depends upon the size, occupation and other activities of the patient and is subject to wide variation. For the average city dweller whose occupation is sedentary and whose hobbies are moderate, diets varying from 800 to 1,200 calories usually work out well.

Some strategy must be exercised in prescribing a diet to individuals who have repeatedly "tried dieting" and have failed to lose weight. In such instances it is encouraging to the patient to start with a very low calorie diet to induce a sharp weight loss. Later adjustments can be made so that they are losing more conservatively. Often it is useful to prescribe definite menus, changing them at frequent intervals.

If a patient successfully adheres to some such dietary regimen for about four weeks and experiences the benefits of weight loss, his subsequent course is usually satisfactory. In other words, it takes about one month to re-educate the appetites of most obese patients, beyond which they cooperate with much less effort.

It is good psychology in some to set a goal for the patient with relation perhaps to the waistline, or the number of pounds to be lost over a given period, or the size of the clothing. Determination can be encouraged in a female if her doctor makes the comment that he wants to see her weight down to where she can wear a size 14 dress. It gives her the feeling that he really understands her problem.

The so-called lipophilia, the tenacity with which mobilization of fat is resisted, is associated with a tendency to retain water and salt. Accordingly, some clinicians believe that the reduction of salt and fluid intake is an essential point in the treatment of obesity. Some carry this out to an extreme degree. For practical purposes it is adequate to advise an obese

patient to use salt sparingly and to take fluids in ordinary amounts. Except for variations in weight of short duration, due to temporary water retention, weight loss will accompany the caloric deficit regardless of the variation in salt and water metabolism. To preserve the patient's morale, the doctor should take pains to explain that weight is rarely lost steadily but that the weight curve often will alternate steady plateaus with periods of sharp decline. The weight may remain fixed for as long as two weeks at a time, due to this water retention, despite rigid adherence to diet. Forced diuresis by drugs is unnecessary.

From the standpoint of therapy, there is no endocrine preparation which is really necessary or helpful in reducing patients. A potent hormone introduced into the body is valuable in replacement therapy only. One risks a disturbance in a delicate glandular balance in prescribing hormones to obese people, a risk hardly justified in view of the poor response in weight loss.

Thyroid hormones should be used only in those obese patients showing definite evidence of hypothyroidism. When thyroid is given to patients with normally functioning thyroid glands, there is a diminution of thyroid hormone production to compensate for the excess being supplied from the outside. When given, the dosage should be adjusted only to improve the signs and symptoms of hypothyroidism without special regard to the weight. It will often elevate the basal metabolism to normal, thereby broadening the caloric deficit on a limited caloric intake. In this way, however, it constitutes only a small aid to weight loss, the main factor in treatment being the diet.

There is no known anterior pituitary preparation which is effective in the treatment of obesity. The use of the thyrotropic hormone and Collip's metabolic principle would be rational in special cases but these substances are not available for clinical use. Neither is there any definite evidence that ovarian preparations are useful in this regard. An obese patient, however, showing definite signs of the menopausal syndrome should be managed with effective estrogenic therapy; her improvement will enable her to cooperate more fully with the weight-losing measures.

Amphetamine sulfate and related compounds are employed in the treatment of obesity because they are claimed to have a calorigenic effect and also because of their ability to produce anorexia or loss of appetite. Well controlled studies, however, fail to reveal any definite benefit traceable to use of these compounds. They should not be used in obese patients except when there are other definite pharmacologic indications.

Diuretics such as the organic mercurials or ammonium chloride have been used in obese patients on the theory that water retention is a contributing factor to overweight. In the usual forms of obesity the use of diuretics or strict restriction of fluids is unnecessary, as already stated.

Laxatives may be required to counteract the constipation so often complained of at the beginning of dietary treatment. Bland non-irritating laxatives should be prescribed. The use of strong saline laxatives to induce dehydration, however, is improper.

Many believe that exercise is the best way to reduce. Actually it is a poor substitute for proper diet. A man weighing 250 pounds must climb twenty flights of stairs to rid himself of the energy equivalent to one slice of bread. In walking he may dissipate 100 calories per horizontal mile, but by omitting an ounce of cream from his diet he will reduce the inflow of calories to the same extent. He must walk 36 miles to rid himself of one pound of fat. Football players do lose 4 or 5 pounds during a game but this is the loss chiefly from perspiration. Active exercises, however, are a valuable though minor accessory in the management of obese patients. They should be encouraged to spend time outdoors in long walks and to do daily general body building and posture exercises. It is possible through vigorous activity to build up muscular tissue while destroying adipose tissue.

Massage is helpful in forming subcutaneous tissue and in helping skin retraction during the process of losing weight. But it is of little value in actual reduction. The only person who loses weight through massage is the masseur. Turkish or cabinet baths produce a temporary loss of water, of extremely limited value. They are hardly worth the effort and risk.

Marked weight loss, particularly in middle aged and older patients, may be accompanied by redundancy of the skin in various parts of the body. There may be a persistent so-called abdominal apron or loose wrinkled skin about the neck, for which plastic surgery is desired. There should be a delay of a year or two before plastic procedures are done in order to allow

maximum spontaneous skin retraction and in order to be sure that the patient stays normal in weight.

DR. READER: Thank you, Dr. Melchionna. Dr. Hinkle, I wonder if you could give us any further ideas as to treatment of the psychologic problems in obesity?

DR. LAWRENCE E. HINKLE, JR.: The people who are overweight are those who consistently, over long periods of time, take in more food than they utilize. The usual reason they do this is simply that they enjoy eating. Not only do they get satisfaction out of a good meal but also, when things are going badly for them, when they are under pressure or tension, they eat more because it makes them feel better. This group of people nearly all recognize the pleasure which they get out of eating and the difficulty which they have in controlling their appetites. Since most of them are otherwise organically normal, the problem is that of helping them control their appetites. In this sense psychotherapy belongs in the hands of the family doctor. One of the things that the doctor can do is to give these people support and encouragement in a field in which they are notoriously weak-willed. In addition to giving them a diet, he can interest them in various forms of group reduction classes, charm classes or dietary groups. These converge the patient's activities and interests into a group which gives him the support of other people with a similar problem. This procedure is used a great deal by commercial reducing establishments. These so-called salons enroll their clients in groups and work to develop a group spirit and morale. I would guess that their methods are more effective than our own attempts to do the same thing on an individual basis with each patient. Likewise the doctor may prescribe some medication, such as amphetamine, ostensibly for the purpose of reducing appetite although, as Dr. Melchionna has stated, the degree to which these medications reduce appetite seems to be pharmacologically small but psychologically large. The important point is to recognize that the prescription of a pill to reduce appetite is a reasonable form of psychologic support, so long as the doctor does not prescribe some medicine which does more harm than good.

One must in obese persons attempt to discover the gratifications which they have lacked in their lives, be it a question of loneliness, too much pressure of business, some easily adjustable family difficulty. By relatively simple manipulations, such as suggestions for other activities or changing of one's job, the doctor may help an individual to relieve himself of the tensions which generate an increased appetite. Simply finding a sympathetic listener in his doctor is of great benefit to a fat man.

That I think covers the major proportion of obese people and I believe that such therapy definitely belongs in the hands of the practitioner. There are only two groups of obese people who, to my mind, need to be in the hands of a psychiatrist. One group I can mention only in passing; those obese individuals whose psychologic problem is of the severity of a psychotic illness. They must be recognized as such and placed under psychiatric care. The other group which demands more intensive and specialized care contains the young obese people, particularly the very fat adolescent girls and young women. Psychologically they are people who have a very intense urge to put things into their mouths. They fall into the broad group in which we also find people who are alcoholics, people who smoke a great deal, people who have anorexia nervosa. In these cases one usually finds that early in their lives they suffered what they believe to be repeated deprivations. This is usually centered around their relationship to a mother whom they found to be unsatisfactory. So they developed the substitute gratification of eating to a degree which they cannot control. The treatment of each case, as in all psychotherapy, has to be tailored to meet the needs and problems and personality structure of the individual patient, and I don't think that we ought to go into that at this time.

DR. READER: I was interested in your remark, Dr. Hinkle, about the serious implications of obesity in adolescence. I have heard it said that the problem is even greater in young males than it is in girls. Supposedly the onset of obesity at puberty carries greater indications of psychopathology, and the prognosis for weight reduction is poorer in males than in females. Perhaps we could hear from Dr. Harvey about that.

DR. WILLIAM A. HARVEY: Well, the matter of becoming too fat is a problem to a growing boy in many ways. Particularly it raises doubts in his mind of his own manliness. I think that in such cases doctors are apt to accept obesity as a failure of maturation and to believe that food restriction is hence doomed to failure. But I don't see that there is any sex difference; I

don't know of any reasons why it should be considered more serious in boys than in girls.

DR. READER: Dr. Korsch, I wonder if you think it is more difficult to treat obesity in children than in adults.

DR. BARBARA M. KORSCH: I think it is. We are singularly unsuccessful in the Children's Clinic in treating obesity because we have a more complex problem. In younger children one has to deal with both the mother and the child because the amount of food intake is often not determined by the child but by the mother's need to feed the boy or girl. In such situations the mother is usually unable or unwilling to enforce a diet. Then, too, it is the mother who thinks the child should be reduced but the child has no such urge.

DR. READER: The direct approach to these problems always appeals to us and Dr. Conway, as a surgeon, has the most direct attack of all. So we will ask him how he goes about handling obesity.

DR. HERBERT CONWAY: I would like to speak of only one aspect of obesity, regional accumulation of fat. In the Plastic Surgery Department of the New York Hospital approximately fifty patients who ask for the excision of localized accumulations of fat, obviously dietary in origin, are examined in the course of one year. Our records show that only four are approved each year for surgery.

Lipectomy is considered for accumulations of fat in the neck, legs, abdomen, and occasionally in the renal region. In selected cases the operation is not to be frowned upon. It has been our experience that when the patient has stabilized his weight under competent dietary supervision but is left with fat hanging in a pendant apron, the fat can be successfully excised with its overlying skin without concern as to recurrence of that localized apron. The neck and the abdomen are most frequent sites of complaint, especially in older people. The abdominal aprons may be completely disabling, if only because of the severe dermatitis which develops on the adjacent surfaces of skin under such an apron. The operation for removal of loose folds of skin in the neck is known as rhytidectomy, from the Greek root "rhytis" meaning wrinkle. It is commonly referred to as the "face lifting" operation. It is my opinion that no operation in the field of surgery is more widely abused than this particular procedure. In my own experience I meet at least 100 patients a year who ask for this neck operation, but anyone who consults the records of this hospital will find that no more than four or five patients are subjected to rhytidectomy during any given twelve-month period. The operation is not at all a difficult procedure. It is not one in which the average surgeon is interested because it is a rather monotonous mechanical procedure. The reason that so few patients are approved for the operation is that in the vast majority of cases a significant improvement cannot be achieved. A lady in her sixties, who has been thin most of her life but has loose folds of skin in the neck, will achieve a dramatic result following incisions in front and back of the ear through which large sections of skin are removed. The incisions can be sutured so that they are almost invisible. These cosmetic operations often heal without perceptible scar. But most of the people who want the operation are women in their forties, with rather moon-shaped faces and thick subcutaneous fat deposits in the submandibular region. They are not the type for which the operation will produce the desired improvement in appearance.

In association with the pendant fat on the abdomen, very often there is diastasis of the rectus muscles or ventral hernia. This is understandable in that these individuals have excessive accumulation of omental fat, too. When such people achieve proper loss of fat generally but the apron is left, we approve excision of the apron through an incision which may be as much as 36 inches in transverse dimension. Usually we take out a huge section of skin and fat. To our disappointment such a specimen may weigh only five or six pounds. At the time the abdomen is open, the excess omental tissue is excised, and the diastasis recti or ventral hernia is repaired. The procedure may be highly successful in selected cases.

I failed to mention one other condition. That is the ptosis of the breast which is due in part to accumulation of fat in the mammary structure. In those patients, just as in those with the large panniculus adiposus of the abdomen, the resection of the breast by breast plasty may be successful.

Microscopic examination of the cells in abdominal aprons of fat has indicated that they are in a mild state of passive congestion. There may be minute hemorrhages in the skin. The skin very often is edematous and shows the linear striae which are common in people who

gain weight rapidly. They are due to tears of the dermis of the skin caused by overtaxing of the elastic fibers. The epithelium remains intact so there is no overlying open wound. The scarification under the epidermis gives the picture of scar epithelium just as though there had been an open wound.

The lipomas, which are localized collections of fat, are treated successfully by surgery. They also bring up some interesting questions in regard to fat metabolism. There is more involved in the deposition of fat than just the volume of calories taken in. Some hereditary factors may be involved, or at least lipomas may be present at birth. I have seen lipomas of the fingers in an adult associated with bony overgrowth of the metacarpals, an indication that the fat deposits were present during intrauterine life. Lipomas may be symmetrical. Further, I remember an interesting finding in a young boy with a scar contracture of the hand. At the age of fourteen a skin graft was performed, with a flap of skin and underlying fat from the abdomen being transplanted to the hand. Several years later he had become moderately obese, with a good deal of fat on the abdomen. The graft of abdominal skin and fat on the hand showed the same increased depth of subcutaneous fat and stood out from the rest of the hand. Another comment on the irregularity of activity of adipose tissue in the human body is brought out by the fact that a person may come to autopsy in an emaciated condition with practically no subcutaneous fat. Yet there may be a good malar pad in the cheek and suprarenal fat may still be present. So there is a wide variation in the distribution of fat in the human body. Perhaps there are certain specific characteristics of fat metabolism in the different areas of skin and subcutaneous tissue. I think this should be kept in mind and curiosity so directed that some light may be thrown on this subject of regional obesity.

Dr. Frank Ferguson: Does all exogenous obesity necessarily represent some degree of psychiatric disturbance? A person may in his youth form habits of heavy physical labor which necessitate a high caloric diet. Then in middle age, when his activity is reduced, he may continue the same diet through habit and thus become obese.

Dr. Melchionna: That is true. I do not mean to imply that all overweight people have some emotional problem. Theoretically, of course, as one's needs for calories are reduced, the appetite is also reduced and less food is eaten. In actuality the mechanisms governing the appetite do not always work properly. So in some cases habit patterns may be the principal cause of obesity and treatment should be primarily a matter of explanation and institution of new habits, without the need for delving so deeply into the patient's life. I would not want to hazard a guess as to what proportion of obese people were that way simply due to habit, but I would be inclined to think the percentage was small. The problem is rarely that simple.

Dr. Reader: As an incidental point, Dr. Hinkle, what is your opinion of the notion that in an obese patient with early diabetes, or a "tendency" toward diabetes, loss of weight will cause improvement or even an apparently complete remission of the disease?

Dr. Hinkle: I think there is no question that reduction in weight ameliorates the symptoms of diabetes, especially in older patients. It is even possible that the glucose tolerance curve may return toward a normal configuration. However, I am not sure that obesity is the cause of diabetes. There is more to it than that.

Dr. Reader: Dr. DuBois, you have not had much to say so far today. Do you have a statement you would like to make at this time?

Dr. Eugene F. DuBois: I would like to suggest, in connection with the relationship of the endocrine glands to obesity, that everyone read a short article by Fuller Albright in Cecil's "Medicine." It is an introduction to the endocrine system but he discusses chiefly the things that are not endocrinology. It is a very delightful, humorous discussion and throws, I think, a good deal of light on the conception of the causes of obesity.

Then there is another little book that has appeared recently that I suggest also be read. It is in the American Lecture Series on obesity, by Rynearson and Gastineau. It seems to me, that sums up better than any other article or book that I have read the conservative opinion regarding the relationship of endocrine function to obesity and discusses all the other causative factors of obesity. Rynearson and Gastineau are rather anxious to do away with the so-called division between endogenous and exogenous obesity. They think that they are all the same thing, that the so-called exogenous obesity involves the endogenous factor of too much appetite, and the so-called endogenous is related

to the exogenous factor of too much food. So probably it would be better if we gave up that rather old-fashioned and arbitrary division.

I was interested in your statement, Dr. Melchionna, that endocrinopathies probably contributed only about 3 per cent of the obese patients. I wonder if such a statistical relationship could not occur even if there were no causal connections between the two. Such a low percentage could be caused purely by coincidence. After all, patients with endocrine disease can be either fat or thin. I hope that the endocrine factor in obesity will be minimized as you have done in your introductory talk.

DR. READER: Miss Jones, as a dietitian, how do you go about discussing a low calorie diet with a patient?

MISS MEREDITH JONES: There isn't time to go into details of calorie contents of foods. Anyone interested can get the information from a book entitled "Food Values of Portions Commonly Used" by Bowes and Church (published by College Offset Press, Philadelphia 6, Pa.). That is an excellent book. Physicians can also obtain booklets of caloric values from the Metropolitan and other life insurance companies, which are fairly reliable.

I would like to mention a few popular errors in reducing diets. As Dr. Melchionna has mentioned, we recommend liberal supplies of meat and fruit in a diet. However, these foods are not inert. An average serving of fresh fruit contains 40 to 80 calories and canned or frozen fruits have 100 to 200 calories per serving. Thus fruit makes a better dessert than pie or cake, with 200 to 400 calories. But if the patient feels that the fruit is "non-fattening" and so eats more, he is no better off. In regard to meat; pork, ham and bacon must be excluded from the diet. These contain so much fat, even if the excess is trimmed from the outside, that they are of very high caloric content.

Elimination of desserts is a big stumbling block. Patients believe that everything good is being removed from their lives and they don't adhere to the diets. There are some desserts, in addition to fruits, which can be used. Jello, sherbets, custard, Junket and sponge or angel food cake without icing are all of low caloric content. Soft drinks, colas and ginger ale have to be banned as they contain some 150 calories. Club soda, however, has no calories at all.

One point that I don't think is given adequate consideration is the method of preparation of food. The way the food is cooked is tremendously important. I can give you one example in the case of the breakfast egg. An average boiled egg yields about 75 calories but the same egg if fried contains 110. Again, a head of lettuce makes an excellent salad and adds only 10 calories. But a single tablespoonful of Russian dressing runs the caloric content to 110. A physician must look into the use of cooking oils, frying or creaming foods, and addition of bacon fat and oil to vegetables, especially in cases in which the patient may not be losing weight even though apparently on a proper diet. In many cases this will run into racial habits. Jewish people use large amounts of sour cream, cream cheese and chicken fat. Other styles of cooking may also add many calories to the meal.

We try to adapt the reducing diet to the patient's eating habits. If the patient is used to meat in the evening but not at lunch, for example, we leave things that way. If he divides his food intake evenly among three meals, we adapt the diet to this same routine. At times we do go contrary to previous habits for the sake of better balance. A person who skips breakfast often is only fooling himself, as he uses this as an excuse to eat twice as much for lunch. Or he takes a bedtime snack of 500 or so extra calories. If each meal is of a somewhat substantial nature, it is easier to control the appetite and the caloric intake. Sometimes this leads to confusion. People who usually take nothing but coffee for breakfast will think a doctor is crazy if he talks of cutting down the food intake and then suggests eating a slice of toast and some eggs in the morning. This requires explanation.

There is one additional comment I have. We have found that better weight reduction is obtained if the doctor has first taken time to tell the patient why weight must be lost. After the patient knows the reasons and has a definite goal in mind, he is much more receptive to our words about diet. But if the doctor has simply scolded the patient or just told him he needed a diet, the outlook is very poor.

As far as the results that can be obtained, we have compared our figures with the claims made by some of the Fifth Avenue salons. They state that 90 per cent of their clients successfully lose weight. The best that we can do, under optimum conditions, is to take substantial amounts of weight off only 65 per cent of our patients.

DR. READER: Is there any comment on Miss Jones' statement?

DR. MELCHIONNA: It brings to mind a paper, the authors of which I cannot recollect, published about twelve years ago. Some growing rats were given the full food supply at one meal while another comparable group was given the same total amount divided into frequent feedings through the day. The rats that got the food in one feeding gained weight much more rapidly than those that had several small feedings each day. There was never any clear understanding of the factors involved. There is resistance to the breakdown of fat stores in an individual who is starved. In those rats fed only once a day, metabolism may have been reduced in the periods of fast between each meal, whereas the rats fed frequently may have utilized the calories more properly after ingestion. If we are to be guided by that experiment, it seems preferable to give a patient at least three meals a day to supply the total calories allowed.

There is another thought that comes to my mind. Most obese people have very distorted dietary habits. They eat largely concentrated carbohydrates and fats. In many cases it is of benefit simply to change the food intake qualitatively, without particular attention to the total caloric consumption. One group in Chicago has claimed good results simply by re-educating the patients to eat a more normally balanced, less concentrated diet. They let patients eat as much as desired, but only of the proper foods. Apparently it is impossible for them to take in as many calories as previously and hence weight is lost. In some cases this approach is useful, particularly if obesity isn't extreme.

I would like to ask Miss Jones how she obtained the figure of 65 per cent successful weight loss. That would be an unusual therapeutic victory.

MISS JONES: We keep a record of all patients on reduction diets. Of those that were followed for a year, 65 per cent reached the desired weight level and maintained that weight for at least six months.

DR. MELCHIONNA: That is a different matter. You are referring to those patients who are followed for at least one year. In this group 65 per cent actually represents a low figure. Most patients drop out in the first month. Actually, if one considered the total number of patients in whom weight loss is attempted, success would be achieved in probably less than 15 per cent through the clinics.

MISS JONES: I am sure that is about the right figure.

DR. MELCHIONNA: There is another dietary fallacy, besides those you mentioned, that has grown up about coarse cereals and whole wheat bread. It is a good principle to substitute whole wheat for white bread when putting a patient on a diet, simply because the whole wheat will furnish more essential nutrients, but both types of bread can be fattening. The patient who substitutes six slices of whole wheat bread for his usual six slices of white is not going to lose weight.

MISS JONES: The caloric content of both kinds of bread is identical. People also have the idea that toasting cuts down the calories, which just is not so.

DR. WALTER MODELL: I wonder if I am repeating Dr. Hinkle correctly. He agreed with Dr. Melchionna that drugs such as amphetamine exerted effects on appetite only through psychologic means. But I gathered that use of such drugs was effective and justified in his opinion.

DR. HINKLE: I did not necessarily advise that amphetamine might be used, although it is now a popular drug, and if given in not too large doses is harmless. The problem with most obese patients is their need for help in overcoming the urge for food. If they get some psychologic support from a medication prior to meals, which they think will reduce their appetite, I do not believe that is a bad thing.

DR. MODELL: I am not taking exception. My point was solely in regard to use of amphetamine for such a purpose. There are distinct dangers in using that drug and I wonder why you would not just prescribe a placebo.

DR. HINKLE: There are indeed some dangers in using the drug. But a placebo is effective only in so far as it inspires the confidence of the patient.

DR. MELCHIONNA: I would like to support your point, Dr. Hinkle, which I think is a good one. The prescription of drugs, particularly if they are to be taken before meals, is good practice. Just the act of taking a pill before a meal is a reminder to the patient to eat less at that meal, in addition to any effects, psychologic or not, on the appetite. The vitamins administered with a low caloric diet serve this function. The same is true of amphetamine. I am not impressed with the dangers of this drug in usual doses, such as 5 mg. at a time. The patient may have some stimulatory effects from this dosage;

he may have some difficulty in getting to sleep at night. For this reason, only two doses are often given, before breakfast and before lunch.

DR. MODELL: That practice is perfectly all right. But I still object to the usage of amphetamine without definite indication.

DR. HINKLE: I did not mean to imply that amphetamine is the only drug to be used. It may actually have no more physiologic effects than any other placebo. But it must be recognized that many doctors believe it has definite anorexic properties and the public is aware of this. A physician should not scorn these psychologic factors.

DR. SOLOMON GARB: What is the composition of the reducing tablets advertised so much today?

DR. MELCHIONNA: Many of the advertised remedies are mixtures of vitamins, minerals and egg or milk proteins. They contain very few calories. I believe the important thing is that they are supposed to be taken before meals. The patient feels hungry and takes a tablet. Psychologically that might alleviate hunger.

DR. GARB: Do you restrict alcohol in a reducing diet?

DR. MELCHIONNA: Yes. There is some confusion about the utilization of alcohol. It is true that alcohol cannot be deposited as fat nor consumed for work energy. But it can be metabolized to furnish body heat and thus can spare other calories which will then be laid down as fat. So I insist that the patient eliminate alcoholic drinks.

MISS JONES: Liquors contain even more calories than those due to alcohol. A glass of beer contains 120 calories, and sweet wine has 160 per wineglass. Cocktails run about 140 to 170 calories each. Probably the top caloric content is in Christmas eggnog. The usual punch-cupful of this runs in the neighborhood of 330 calories.

SUMMARY

DR. FERGUSON: In regard to the cause of obesity, the opinion of this conference seems flatly opposed to the belief that obesity is usually related to diseases of endocrine glands. In only rare instances is an endocrinopathy found in obese persons and in such cases the glandular disorder is associated more with the distribution of fat than with the total weight. Instead the emphasis has been on psychologic factors as a cause of overweight. Such factors may range from a fairly simple problem in life to a defect in personality or even to an overt psychosis. All these may result in increased food consumption and hence obesity.

The foundation of treatment for obesity is diet. We have heard discussed the desired caloric content and the composition of a reducing diet and means by which this can be adapted to a patient's habits. In addition attention must be paid to the methods of preparation of food. Various fallacies of reducing diets have also been mentioned. Vitamin supplements should be added to the diet.

Rather simple psychotherapeutic measures have been outlined. It is necessary for the doctor to give the patient a thorough understanding of the dangers of obesity and the advantages to be gained by weight loss. Since therapeutic success is dependent upon the patient rather than the doctor, maintenance of patient morale is of the utmost importance. The use of placebos has been recommended. The physician must also attempt to uncover the psychologic disorders responsible for development of obesity. In many cases these problems will be easily corrected, and only rarely will specialized psychiatric care be needed.

Plastic surgery seems of rather limited use, applicable more to the sequellae of weight reduction than to the obesity itself. It is important, before considering surgery, that the weight be brought near the desired level and that a sufficient interval elapse to ensure maintenance of weight loss and maximum retraction of pendant tissues. Active exercise and passive massage may also be of benefit in controlling these sequellae, although exercise in any form is of limited value so far as losing weight is concerned.

Medications have practically no place in the management of overweight persons unless there is some associated disease which also requires therapy. Endocrine preparations, including those of the thyroid, pituitary and ovary, are of no value in obesity and may even be dangerous. Amphetamine, though reported to produce anorexia, is regarded as of questionable value. Diuretic drugs have no place in the therapy of obesity. There may be variations in salt and water retention which temporarily hinder weight loss but the use of diuretics or restriction of salt or water serves no useful function. The only drugs recommended are the addition of vitamin supplements to the diet and the use of any harmless drug before meals as a placebo.

Clinico-pathologic Conference

Leukemoid Reaction, Abdominal Pain and Mesenteric Lesions

STENOGRAPHIC reports, edited by Robert J. Glaser, M.D. and David E. Smith, M.D., of weekly clinico-pathologic conferences held in the Barnes Hospital, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient, G. M. (No. 7740), was a sixty-seven year old white barber who entered the Washington University Clinics on August 22, 1951, complaining of weakness and pains in the arms and legs. The family history was not contributory. The past history was of interest in that at the age of twenty-five the patient developed an enlarged gland on the left side of his neck. Although he was told it might represent a tuberculous lesion, it was not treated and cleared without further event. Five years before admission the patient developed bilateral inguinal hernias. The past history and systemic review were otherwise negative.

For about one year before coming to the clinic the patient was troubled by increasing fatigue which six months before entry became marked. He also had "aching sensations" in both his arms and legs; the latter symptoms were at times extremely painful, particularly at night. Three weeks prior to admission, while at work, the patient fell to the floor but apparently did not lose consciousness. He was seen by a physician who told him that he had "anemia," for which injections of liver, iron and vitamin B complex were given. In the following three weeks he was unable to work because of weakness but he did not remain in bed.

When seen in the Washington University Clinics, he was afebrile. The significant physical findings were pallor of the skin and bilateral inguinal hernias. The laboratory data included a red cell count of 3,500,000 with 12 gm. of hemoglobin, a white cell count of 25,000 and a normal differential. Gastric analysis revealed no free acid in a fasting specimen, but free acid was present after the injection of histamine. The patient was admitted to the Barnes Hospital on August 25, 1951, for further study.

At the time of entry into the hospital, the physical examination revealed the patient's temperature to be 38°c., pulse 20, respirations 24 and blood pressure 180/115. He was pallid and appeared chronically ill. The skin was of yellowish cast but the conjunctive were not icteric. Examination of the fundi revealed only arteriolar narrowing. Examination of the upper respiratory tract was negative except that the tongue was somewhat redder than normal. The lungs were clear to percussion and auscultation, and the heart was normal. Abdominal examination revealed no organs or masses. The inguinal hernias previously noted were present and easily reducible. Rectal examination was negative. The neurologic findings were normal except for absent vibration sense in the left leg and foot.

The laboratory findings were as follows: *Blood count:* red cells, 3,480,000; hemoglobin, 11.6 gm.; white cells, 25,300; differential count: basophiles 2 per cent, eosinophiles 4 per cent, myelocytes 1 per cent, juvenile forms 8 per cent, stab forms 12 per cent, segmented forms 59 per cent, lymphocytes 7 per cent, monocytes 7 per cent; reticulocytes, 5.7 per cent; platelet count, 280,000. *Urinalysis:* negative. *Stool examination:* negative. *Blood cardiolipin test:* negative. *Blood chemistry:* non-protein nitrogen, 13 mg. per cent; sugar, 101 mg. per cent; phosphorus, 4.2 mg. per cent; acid phosphatase, 1.3 K.A. units; alkaline phosphatase, 5 Bodansky units; chlorides, 91 mEq./L. *Basal metabolic rate:* +20, +27 per cent. *Roentgenogram of the chest:* The aorta was lengthened and tortuous. Calcified hilar and cervical nodes were present and there was a primary Ghon complex in the left upper lobe. The gastrointestinal series revealed a traction diverticulum of the lower third of the esophagus. Metastatic bone series showed narrowing of the interspace between the fourth and fifth lumbar vertebrae with hypertrophic changes. *Electrocardiogram:* left ventricular strain.

Soon after the patient was admitted to the

hospital, bone marrow studies were performed. The marrow was found to be cellular with a moderate predominance of myeloid cells and some shift to younger forms. The erythroid elements were present in normal numbers, but also showed a slight shift to younger forms. Megakaryocytes were plentiful. No tumor cells were noted. A definitive diagnosis could not be made on the basis of the bone marrow studies. A first strength PPD skin test was negative. During his hospital stay the patient's blood pressure fell to normal value; his temperature ranged between 37.4° and 38.0°C. He was discharged from the hospital on September 6, 1951, to be followed in the clinic.

For the first week following discharge from the hospital his condition remained unchanged. Eight days prior to the second admission he vomited once and on the following day developed severe upper abdominal pain which radiated to the right costal margin. He was seen by a physician who gave him a hypodermic injection and the pain subsided to some extent; it continued to be present constantly, however, during the week prior to the second admission. His afternoon temperature ranged between 99° and 100.4°F. His skin became yellow, the urine dark and the stools light in color. He had no further vomiting and noted no change in the character of the stools. Although the patient's abdomen did not become distended, for several days prior to entry the right inguinal hernia could not be completely reduced. Because of the continuation of his symptoms he was admitted to the surgical service on September 21, 1951.

Physical examination at the time of entry revealed the temperature to be 38.3°C., pulse 100, respirations 22 and blood pressure 140/80. The patient had obviously lost weight. The skin and sclerae were definitely icteric. Examination of the eyes was not remarkable and the upper respiratory tract appeared normal. Matted lymph nodes of unstated dimensions were palpable in the left supraclavicular fossa and firm inguinal glands, 1 to 2 cm. in size, were palpable bilaterally. Examination of the heart and lungs was within normal limits. There was tenderness on deep palpation in the epigastrium and in the right upper quadrant; in the latter area a firm tender mass extending 5 cm. below the costal margin was felt. It apparently was situated superior to the liver since the liver edge was also palpable beneath the mass. No other abdominal viscera were felt. The right inguinal hernia could not be completely reduced. The neurologic examination was within normal limits.

Laboratory findings were as follows: *Blood count:* red cells, 3,250,000; hemoglobin, 11 gm.; white cells, 60,000; differential count: basophiles 1 per cent, eosinophiles 2 per cent, myelocytes 16 per cent, juvenile forms 11 per cent, stab forms 26 per cent, segmented forms 39 per cent, lymphocytes 5 per cent. *Urinalysis:* albumin, 1+; sugar, negative; urobilinogen, positive 1/10, negative 1/20; sediment, negative. *Stool:* guaiac positive. *Blood chemistry:* non-protein nitrogen, 19 mg. per cent; sugar, 95 mg. per cent; chlorides, 114 mEq./L.; sodium, 141 mEq./L.; potassium, 5.3 mEq./L.; cholesterol, 123 mg. per cent; total proteins, 6.5 gm. per cent; albumin, 4.2 gm. per cent; globulin, 2.3 gm. per cent; cephalin-cholesterol flocculation test, 3+; thymol turbidity, 1.5 units; icterus index, 8 units; bromsulphalein dye retention, 36 per cent in forty-five minutes; alkaline phosphatase, 11 Bodansky units; sodium bilirubinate, 0.76 mg. per cent; bilirubinglobin, 1.36 mg. per cent; prothrombin time, 57 per cent of normal. *Roentgenogram of the upper gastrointestinal tract:* there was posterior displacement of the stomach due to enlargement of the liver. Prompt but incomplete filling of the duodenal bulb was noted; filling was presumably limited by extrinsic pressure of a mass in the right anterior aspect. It was thought that the mass might represent the gallbladder although it could not be visualized on oral cholecystograms.

Shortly after admission to the hospital a second stool specimen was positive for occult blood. Bone marrow aspiration was performed and revealed some shift to the left in myeloid elements with normal erythroid elements. Gastric analysis showed free acid after histamine; the cell block section of the gastric washings was negative. The patient continued to have a leukocytosis and low-grade fever with temperatures ranging between 37° and 38.5°C. Pain and tenderness in the right upper abdominal quadrant remained pronounced; and because a specific diagnosis could not be made, the patient was subjected to exploratory laparotomy. The mass in the right upper quadrant was found to be a thickened, distended gallbladder. The liver appeared grossly normal although it was perhaps slightly enlarged. The spleen was twice normal size. In the mesentery of the small bowel were multiple, plaque-like

areas of indurated tissue measuring 1 to 7 cm. in diameter. The largest lesion was approximately 1½ cm. thick. The involvement was generalized in the mesentery of the small bowel; and although the sigmoid mesentery was not visualized, it was palpated and thought also to be involved. The gallbladder was removed; its contents consisted of black necrotic material and a few stones. There was marked induration and edema in the region of the cystic duct. The common duct was not dilated but it was not possible to probe it. Biopsy of the mesentery of the small intestine was taken. During the exploration a thick-walled mass was felt along the course of the right ureter. It appeared to contain clear fluid and was removed. The surgeon concluded that the latter mass was an incidental finding unrelated to the patient's primary disease.

The postoperative course was initially uneventful but on the third day the patient developed marked abdominal distention. A diagnostic paracentesis was performed and bloody fluid obtained. The patient's red blood cell count fell to 2,700,000 and the hemoglobin to 8.9 mg. per cent. Similar counts were obtained on the paracentesis fluid. The platelet count was 17,000, the clotting time normal and the bleeding time eleven minutes. The patient was given three units of whole blood and his hematocrit rose from 27 to 35 per cent. Bone marrow aspiration was again performed and revealed questionably abnormal megakaryocytes. Splenectomy was considered but it was decided that the patient's condition was too critical to permit operation, and he was transferred to the Medical Service on October 2, 1951.

At the time of transfer he appeared acutely ill. Breath sounds were decreased at the left base and crackling rales were heard there. Except for tachycardia, the examination of the heart was normal. The blood pressure was 160/110. The abdomen remained markedly distended, and there was some oozing of blood through the paracentesis wound. Protamine sulfate, intravenous ACTH and oral cortisone were begun. The patient received ACTH throughout the remainder of his hospital stay. On the fourth postoperative day fine rales were heard at both bases. The venous pressure was 176 mm. of saline, and the circulation time 18 seconds with decholin. The non-protein nitrogen rose to 50 mg. per cent and the urine revealed 2+ albuminuria. The patient was digitalized.

Oozing of bright red blood through the abdominal wound persisted. The hemoglobin fell and transfusions were again given. The platelet count rose to 36,000; the white blood cell count was 78,000 with a marked left shift. Bone marrow aspiration was again performed and cells suggesting those of reticulum cell sarcoma were noted. It was not believed, however, that this diagnosis could be made unequivocally. On the sixth postoperative day, October 7, 1951, the patient developed lower abdominal pain and marked tenderness in the left lower quadrant. His abdomen became more distended and tense, and the right inguinal hernia became completely irreducible and extremely painful. The non-protein nitrogen was 85 mg. per cent. At the end of that day the patient's blood pressure fell to shock levels. His respirations became labored and despite emergency measures he died. During the period he was observed on the Medical Service the white blood cell count never fell below 26,000 and on one occasion reached 90,000.

CLINICAL DISCUSSION

DR. HARRY L. ALEXANDER: By way of recapitulation, it may be well to review some of the major features of the case. This man was sixty-seven years old and came to the Clinics originally because of weakness and pain in the arms and legs of about one year's duration. He was found to have moderate anemia, a leukocytosis and bilateral inguinal hernias. He entered the hospital and the findings noted in the Clinic were confirmed. Although a number of other diagnostic procedures was done, the diagnosis of the patient's major disease remained obscure. Soon after discharge he developed severe abdominal pain and was noted to have a mass in the right upper quadrant. He reentered the hospital and was subjected to laparotomy; a thickened gallbladder containing necrotic material and small stones was found. In addition, a number of small plaques were present throughout the mesentery. These lesions were biopsied but for purposes of discussion, we are not informed of the interpretation made in the Surgical Pathology Department. Dr. Wilson, would you care to comment on the x-ray findings.

DR. HUGH M. WILSON: As was noted in the protocol, films of the chest were not remarkable for a man of this age. The aortic knob was prominent, but the heart was within normal limits as to size and contour. A gastrointestinal

x-ray examination was then made. A diverticulum in the lower third of the esophagus was demonstrated but no other abnormalities of the esophagus or stomach were found. The duodenal bulb appeared normal but on several films there was a suggestion of an extrinsic pressure defect. When the examination was repeated, the defect was more prominent. The remainder of the intestinal tract appeared normal; we did not consider the spleen to be enlarged. A metastatic series was made but was unrevealing. Cholecystograms showed non-visualization of the gallbladder. When the patient returned to the hospital the second time, not only was the extrinsic pressure on the duodenal cap demonstrated, but also it was noted that the lesser curvature of the stomach was displaced to the left. It was our opinion that the liver was enlarged and that possibly the gallbladder was responsible for the pressure defects involving the stomach and duodenum, although as noted, the gallbladder could not be visualized on cholecystography. There was some abnormality in the small intestinal pattern suggesting segmental areas of spasm but this finding was not striking.

DR. ALEXANDER: Can the findings which you have enumerated be correlated with the multiple mesenteric lesions noted at operation.

DR. WILSON: Perhaps one could consider them to be related to the abnormal small intestinal pattern.

DR. ALEXANDER: Do you think that they suggest intrinsic disease of the intestine?

DR. WILSON: Similar changes are sometimes seen in carcinomatosis of the peritoneum and they can also be seen with inflammatory processes. As a matter of fact, we usually use a rather non-specific term, irritation pattern, to describe the finding noted here.

DR. ALEXANDER: I should now like to turn attention to a consideration of the hematologic findings. At one point the question was raised as to whether the patient might not have myeloid leukemia. Dr. Harrington saw him and did not think that was likely. Dr. Harrington, is your opinion still the same?

DR. WILLIAM J. HARRINGTON: We were, of course, bothered by the possibility that this man might have leukemia, Dr. Alexander, but we never considered it the most likely diagnosis. In general, the presence of anemia and of thrombocytopenia, both of which this patient exhibited, are somewhat uncommon in early myeloid leukemia. Furthermore, none of the physical findings was particularly suggestive of that diagnosis. Aside from the left shift in the differential, we thought it would be difficult to defend the diagnosis of myeloid leukemia. It should also be noted that the absence of basophilia is against the diagnosis of myeloid leukemia.

DR. ALEXANDER: We shall discard myeloid leukemia as a diagnostic possibility. Would you call this a leukemoid reaction, Dr. Harrington?

DR. HARRINGTON: I think that one may use the term leukemoid reaction when the white count reaches levels of 40,000 to 50,000.

DR. ALEXANDER: Infections of various types are among the possible causes of leukemoid reactions. Dr. Harford, do you think this patient might have had tuberculosis?

DR. CARL G. HARFORD: I am inclined to doubt it.

DR. ALEXANDER: You will remember that at the age of twenty-five he was supposed to have had a tuberculous gland in the neck. Why do you doubt that he had tuberculosis?

DR. HARFORD: I would have expected the mesenteric lesions to involve the lymph nodes primarily had the process been tuberculous in origin.

DR. ALEXANDER: Does the fact that the patient had extensive mesenteric lesions without pulmonary involvement militate against the diagnosis of tuberculosis?

DR. HARFORD: In this country bovine tuberculosis has been essentially eradicated and intestinal tuberculosis due primarily to the ingestion of tubercle bacilli from bovine sources is quite rare. On the other hand, tuberculosis of the intestines, secondary to pulmonary tuberculosis, certainly occurs. Therefore, the fact that the patient's chest film was negative does indeed make tuberculosis an unlikely possibility.

DR. HAROLD SCHEFF: In addition to the negative chest x-ray, the patient also had a negative first strength PPD test which is against the diagnosis.

DR. ALEXANDER: Would you comment, Dr. Harford, on the value of a negative first strength PPD in ruling tuberculosis in or out.

DR. HARFORD: In my opinion, Dr. Alexander, a negative first strength test merely suggests that it is safe to go ahead and use the second strength preparation. I do not believe it is of much significance otherwise. If the second strength test is also done and is negative, that result constitutes definite evidence against the diagnosis of tuberculosis. It is almost unnecessary to point

out that a positive test, *per se* does not substantiate the diagnosis of active tuberculosis.

DR. ALEXANDER: Are there further comments?

DR. THOMAS H. HUNTER: While we are discussing negative PPD skin tests, it might be proper to mention Boeck's sarcoid as a possibility here.

DR. ROBERT J. GLASER: The history of arthralgia-like symptoms and the presence of intra-abdominal lesions of possible lymphoid origin bring to mind Whipple's disease, although I suspect that many of the findings cannot be explained on that basis.

DR. SCHEFF: I do not think it conceivable that this patient had Whipple's disease.

DR. ALEXANDER: Since the patient's diagnosis is relatively obscure, we must include many diseases in the differential diagnosis. Do you think the patient had a malignant tumor, Dr. Mendeloff?

DR. ALBERT I. MENDELOFF: If the patient had malignant disease, lymphosarcoma rather than carcinoma seems the more likely to me. One might have expected that either lymphosarcoma or carcinoma cells would have been found in the bone marrow in view of the leukemoid reaction. Further, carcinomatous involvement to this degree should also have involved the liver and should have produced ascites.

DR. ALEXANDER: The diagnosis of carcinoma probably can be excluded for the reasons enumerated. Dr. Shapleigh, may lymphosarcoma be associated with a leukemoid reaction in which the predominant cells are granulocytic?

DR. JOHN B. SHAPLEIGH: Yes, it may.

DR. ALEXANDER: What about Hodgkin's disease, Dr. Mendeloff?

DR. MENDELOFF: I think that lymphosarcoma is more likely than Hodgkin's disease.

DR. ALEXANDER: Do you think the masses described were mesenteric lymph nodes?

DR. MENDELOFF: I don't know. The description given in the protocol does not make clear what they were; apparently they were not related to blood vessels either.

DR. ALEXANDER: I think it reasonable to assume that the mesenteric masses were lymph nodes.

DR. MENDELOFF: If so, they would be compatible with lymphosarcoma. I cannot, however, fit the stone-filled, necrotic gallbladder into the clinical picture of lymphosarcoma.

DR. ALEXANDER: I assume then that lymphosarcoma would account for all of the clinical findings except the necrotic gallbladder. Dr. Shank, what is your opinion?

DR. ROBERT E. SHANK: Hodgkin's disease actually seems more likely to me than lymphosarcoma. I am not at all sure that this patient had any hepatic involvement.

DR. ALEXANDER: You will recall that the cephalin-cholesterol flocculation test was 3+ and that there was 36 per cent retention of bromsulphalein. I presume that these data are not helpful in terms of arriving at a diagnosis.

DR. SHANK: No, I do not think they are.

DR. HAROLD SCHEFF: I doubt seriously that the patient's jaundice was related to his gallbladder disease. It is rare for acute cholecystitis to produce jaundice unless there is associated cholangitis. This patient probably had diffuse hepatic disease as the basis for his icterus.

DR. ALEXANDER: Do you think that the gallbladder disease was an incidental finding or part of the primary disease?

DR. SCHEFF: The most common cause of the gallbladder findings described here is a cystic duct stone. It is conceivable that lymphoma might involve the cystic duct.

DR. ALEXANDER: What significance should be attached to the fact that the gallbladder was necrotic?

DR. MENDELOFF: Before answering that question, Dr. Alexander, I should like to point out that bromsulphalein retention in the presence of fever is very difficult to evaluate. Further, although the cephalin-cholesterol flocculation test was 3+, the thymoid turbidity test was normal. All in all, therefore, the data in regard to liver function are not very helpful. As far as the necrosis of the gallbladder is concerned, there is only one systemic disease of which I am aware that is associated with necrosis of the gallbladder. That disease is polyarteritis nodosa.

DR. ALEXANDER: Your point is well taken, Dr. Mendeloff. We have had several cases of polyarteritis in which gallbladder involvement was present. The leukemoid reaction is consistent with polyarteritis as is the history of myalgia.

DR. MENDELOFF: Abdominal pain also occurs in polyarteritis.

DR. ALEXANDER: The only factor in this case which I find hard to reconcile with polyarteritis is the group of plaque-like lesions in the mesentery. I do not believe I have seen this type of involvement. Are there other comments?

DR. HUNTER: I cannot get enthusiastic about

FIG. 1. The inferior surface of the liver showing the extensive dissection and destruction from the hemorrhage that arose in the bed of the removed gallbladder.

the diagnosis of polyarteritis. I think lymphoma is the most likely possibility. Lymphoma often is a diagnosis of exclusion when an obscure clinical picture such as this man presented cannot be explained in any other way.

DR. ALEXANDER: How would you explain the gallbladder findings on the basis of lymphoma, Dr. Hunter?

DR. HUNTER: I do not believe I could.

DR. ALEXANDER: Dr. Moore, would you comment on the thrombocytopenia?

DR. CARL V. MOORE: This situation was peculiar, Dr. Alexander. The thrombocytopenia must have been secondary since the clinical picture was not that of idiopathic thrombocytopenia. I suspect that it arose on the basis of the primary disease, whether that was polyarteritis, tumor or infection. One other possibility is that the patient had thrombotic thrombocytopenia but that seems less likely. It should be pointed out that splenectomy might well have benefited this patient and prevented some of the terminal hemorrhagic manifestations.

DR. ALEXANDER: It is noted in the chart that splenectomy was suggested but rejected because of the patient's condition.

DR. MOORE: Yes, it was believed by both the Medical and Surgical Services that the patient could not have tolerated splenectomy.

DR. ALEXANDER: Do you agree with Dr. Moore, Dr. Harrington?

DR. HARRINGTON: Yes, I do.

DR. ALEXANDER: Dr. Butcher, would you discuss the operative findings?

DR. HARVEY BUTCHER: The most striking finding at the time of operation was the plaque-like lesions which averaged 4 to 6 cm. in size. The largest one, from which we took the biopsy, was probably 6 to 7 cm. in its greatest dimension. It was at least 1½ cm. thick and involved all of the thickness of the small bowel mesentery. There was no evidence of caseation or of necrosis. The plaques were firm, grey in color and generalized; at the time we thought they most probably represented lymphosarcomatous infiltration. The spleen was enlarged and so was the liver but neither appeared abnormal.

DR. ALEXANDER: It was quite clear that a definitive diagnosis cannot be made in this case without the pathologic findings. The consensus would appear to favor polyarteritis nodosa or one of the lymphomas although no one has been able to relate the gallbladder disease to lymphoma. Polyarteritis would account for all of the clinical manifestations except the mesenteric plaques and I believe that the correct diagnosis rests between those two. Infection and carcinoma both seem less likely.

Clinical Diagnoses: ? Polyarteritis nodosa; ? lymphoma.

PATHOLOGIC DISCUSSION

DR. MICHAEL MCNALLEY: Externally there were some ecchymoses in the skin of the abdomen, particularly around the abdominal incision from which bloody fluid exuded through the drain opening at the lateral end of the incision. The thorax contained 300 milliliters of serosanguineous fluid in each side. The lungs were diffusely reddened and atelectatic, particularly in the lower lobes. In the abdomen there were 2,500 milliliters of fluid and clotted blood. The hilum of the liver, as shown in Figure 1, was torn by hemorrhage which dissected from the bed of the absent gallbladder along the portal veins and into the parenchyma of the liver. The mesentery at the time of autopsy had no areas that could be described as plaques. There were small areas of gray discoloration scattered over its surface which appeared to be foci of fibrosis such as might result from chronic inflammation. The tissues about the site of the biopsy were slightly indurated for 2 or 3 cm. on each side of the incision. In the lower ileum, adjacent to the biopsied site in the mesentery, there was a small infarct of the wall. Distal to the infarct, for the terminal 40 cm., the ileum was thickened and dark red, and the mucosa was covered with an adherent greenish membrane. A few subserosal ecchymoses and a slight amount of fibrinous exudate were present in this

area, but there was no necrosis of the muscular wall.

The liver was enlarged to a weight of 2,400 gm. but showed only slight congestion in addition to the hemorrhage. The spleen was only slightly enlarged and its surfaces were of normal appearance. The mesenteric lymph nodes were also slightly enlarged, but no changes typical of leukemia were present in any of the organs.

DR. FREDERICK G. GERMUTH: In the specimens of the mesentery and gallbladder from the operation there were extensive chronic and acute hemorrhages and distinct lesions of polyarteritis nodosa. In Figure 2 a vessel from the mesentery shows segmental necrosis of the media in the upper central portion of the illustration. There is extensive proliferation of fibroblasts in the intima, and various types of inflammatory cells are present throughout the entire wall of the artery. The same features with extensive fibrosis around the artery and disruption of the elastic lamella are shown in another artery from the same site. (Fig. 3.) By microscopic examination of the postmortem tissues the diagnosis of polyarteritis nodosa was confirmed and additional lesions were demonstrated in the ileum, stomach, liver, pancreas, adrenals and testes. Most of these lesions, however, were either healed or healing.

Since this patient was treated rather heavily for four and one-half days with cortisone and ACTH, the question arises as to the role these two hormones may have played in the healing process. Figure 4 illustrates an artery from the mesentery near the site of the biopsy. There is still the marked fibrosis surrounding and involving all layers of this artery, but there are very few cells in the walls of the artery, and those cells which are present are those of chronic inflammation; there is no acute necrosis or polymorphonuclear leukocytes. This certainly does not represent complete healing of the lesions, and it might be questioned whether the difference between what was seen at operation and what was seen at autopsy was due to a beneficial effect exerted by cortisone. There are both clinical and experimental evidences that suggest cortisone does at least stop the progress of the disease. In a recent report from the Mayo Clinic,[1] two cases were reported in which muscle biopsies showed very active polyarteritis nodosa before treatment with cortisone or ACTH. One patient died three weeks after treatment and the other patient after three months. At autopsy in neither were there any acute lesions; all of the lesions found were in a healed stage. Experimentally in the rabbit cortisone inhibits the occurrence of the lesions of serum sickness which are similar. I think that from this evidence we can say cortisone exerts a beneficial effect on the course of polyarteritis nodosa, but the difficulty in evaluating the spontaneous relapses and remissions of the disease should be borne in mind.

This patient developed pain in the abdomen several days after operation. It may have been related to the anatomic finding of acute hemorrhagic ileitis and the small segment of infarction at the site of the mesenteric biopsy. Figure 5 shows a section of the ileum with this chronic inflammation. There are lesions of polyarteritis nodosa in this tissue; and although the mucosa has sloughed, there is not the ischemic necrosis of all tissues as in an infarct. I would also like to point out that the pain described in the right inguinal region may have been related to the orchitis that was secondary to polyarteritis nodosa.

The terminal event in this case was intraperitoneal hemorrhage that arose from the site of the cholecystectomy. The bleeding started shortly after the operation and continued throughout the postoperative course, for there was gross and microscopic evidence of its beginning organization.

Relative to the hematologic dyscrasia there was marked hyperplasia of the erythroid, myeloid and reticulum cells of the bone marrow, but the number of megakaryocytes seemed reduced. This pleomorphic picture of cellular proliferation is quite unlike the overgrowth of a single cellular type that is found in leukemia. There was extramedullary blood cell formation in the liver, in the spleen, and to a marked degree in the tissues about the kidneys and in the periadrenal fat. The general histologic picture in the liver (Fig. 6) is complicated by the presence of both the extramedullary hematopoiesis and the cells secondary to polyarteritis of the hepatic arteries. The mixed character of this cellular infiltrate distinguishes it from that of leukemia. Figure 7 shows the blood formation in the tissues about the kidneys which is almost identical in its appearance to the bone marrow. The hyperplasia of elements related

[1] BAGGENSTOSS, A. H., SHECK, R. M. and POLLEY, H. F. The effect of cortisone on the lesions of periarteritis nodosa. *Am. J. Path.*, 27: 537–560, 1951.

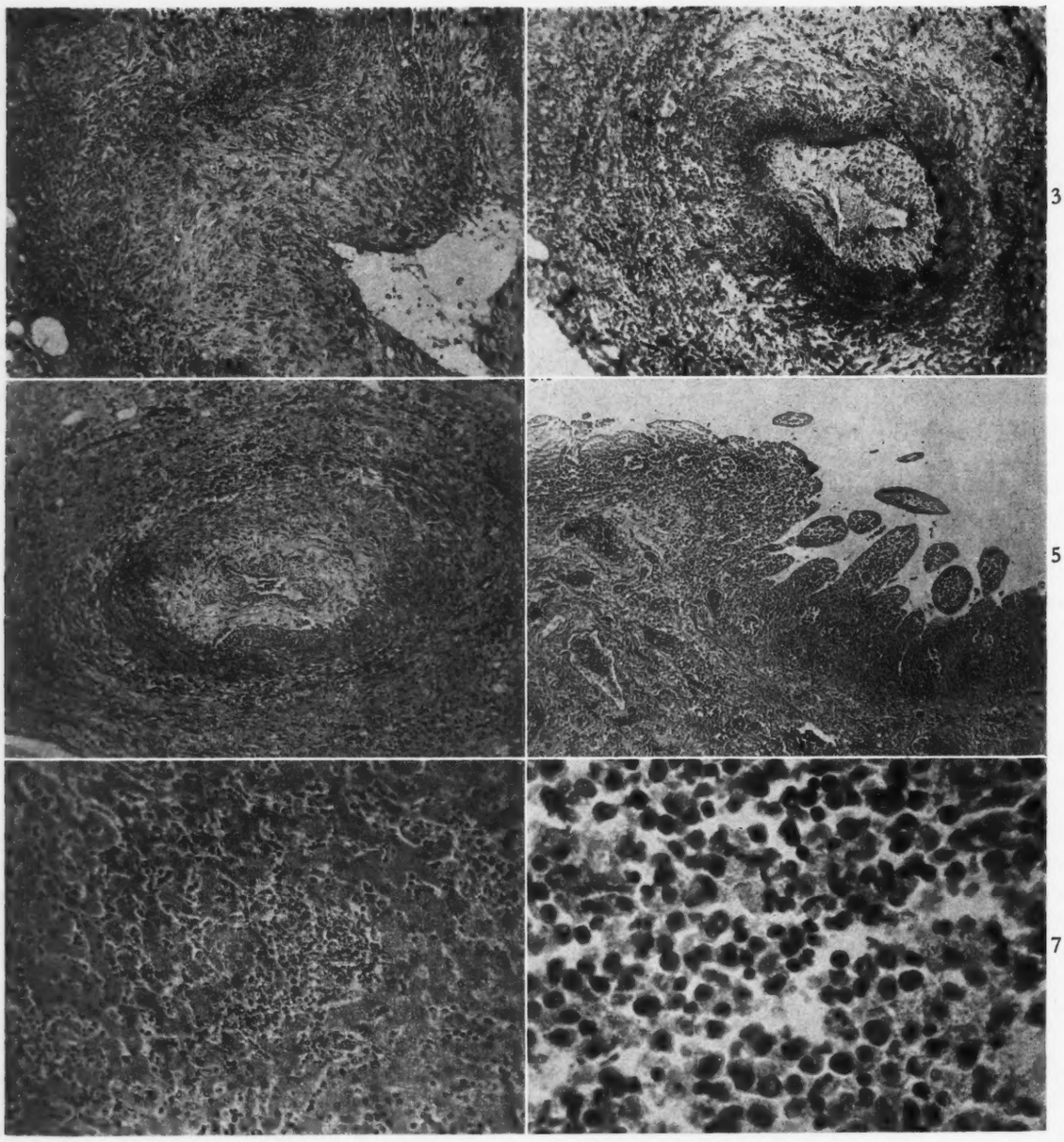

FIG. 2. Segmental necrosis of the media, diffuse infiltration of inflammatory cells and fibrous thickening of the intima in a mesenteric artery affected by polyarteritis nodosa. This lesion was from the biopsy specimen.

FIG. 3. Perivascular fibrosis and disruption of the internal elastic membrane in another artery in the mesenteric biopsy; Verhoff stain for elastic tissue.

FIG. 4. An artery from the mesentery at autopsy showing the persistence of fibrous changes but the absence of necrosis or acute inflammation in its walls.

FIG. 5. The ileum showing sloughing of the mucosa, congestion and chronic inflammatory infiltration but no ischemic necrosis. This is an ileitis rather than an infarct and is related to the lesions of polyarteritis nodosa that were found in this organ.

FIG. 6. Periportal infiltration and cells in the sinusoids of the liver. This pleomorphic cell picture represented a combination of the polyarteritis nodosa of the hepatic arteries and extramedullary hematopoiesis.

FIG. 7. Extramedullary hematopoiesis in the tissue about the renal pelvis. The histologic picture is almost identical to that of the bone marrow.

to the reticuloendothelial system, particularly the reticulum cells, is reminiscent of changes we have recently observed in our experimental animals with polyarteritis nodosa. In the animals there are peculiar lesions in the spleen and lymph nodes that consist of marked hyperplasia of reticulum elements in and about Malpighian bodies and follicles. The Malpighian body is made up essentially of lymphocytes and these elements are made up entirely of reticulum cells. They seem to be a morphologic expression of the immune reaction of the animal to the agent used in inducing polyarteritis.

In conclusion, we have a case of polyarteritis nodosa. There is some evidence that cortisone did exert a beneficial effect on the arterial lesions. There are changes in the body associated with the leukemoid reaction, and the terminal event was intraperitoneal hemorrhage that was in turn probably related to the disordered blood formation.

Final Anatomic Diagnoses: Polyarteritis nodosa with healed and healing lesions in the mesentery, ileum, stomach, liver, pancreas, adrenals and testes; hyperplasia of the erythroid, myeloid and reticulum cells of the bone marrow with reduction in megakaryocytes; extramedullary hematopoiesis in the spleen, liver, parenchyma of the kidneys, tissues about the renal pelves and periadrenal fat; recent and partially organized hemorrhage around the gallbladder bed; hemoperitoneum.

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Research Society Abstracts

American Federation for Clinical Research

ABSTRACTS OF PAPERS PRESENTED AT THE WESTERN SECTIONAL MEETING IN CARMEL, CALIFORNIA, JANUARY 24, 1952

EOSINOPENIC RESPONSES TO VARYING DOSES OF INTRAVENOUS ACTH. *H. W. McIntosh, M.D. (by invitation), R. A. Palmer, M.D., H. S. Robinson, M.D., E. P. Carruthers, M.D. (by invitation) and R. D. T. Cape, M.D. (by invitation).* (From the Vancouver General Hospital, Vancouver, B. C., Canada.)

On continuous intravenous administration of ACTH a characteristic curve was obtained by plotting the numbers of eosinophils found in the peripheral blood against units of time. It was considered that the type of curve obtained might vary with varying doses of the hormone and, if so, in quantitative significance. Six patients were given graded amounts of ACTH over an eight-hour period and the results were plotted on graphs. The results show that, apart from a small range between 0.075 and 0.175 mg./hour, the response is of the "all or none" type. Either there is a complete response as shown by a fall in eosinophils of 80 to 90 per cent in the eight-hour period or there is no significant fall at all. In the range mentioned above, however, there is a suggestion of an increasing response which will be illustrated.

AMIDATION OF GLUTAMIC ACID BY HUMAN BRAIN IN VIVO. *G. S. Gordan, M.D., J. E. Adams, M.D. (by invitation), C. T. Anderson, M.D. (by invitation), E. Eisenberg, M.D. (by invitation), H. Harper, Ph.D. (by invitation) and Q. J. G. Hobson, M.D. (by invitation).* (From the Langley Porter Clinic of the Department of Mental Health, State of California, the University of California, Divisions of Medicine, Neurological Surgery and Psychiatry, and the University of San Francisco, Department of Biology, San Francisco, Calif.)

The original nitrous oxide technic of Kety and Schmidt permits quantitation of the rate of cerebral blood flow in man. Since mixed cerebral venous and arterial blood are sampled, arteriovenous differences can be determined for any substance which may be analyzed in blood. It is therefore possible to measure rates at which the measured substances are utilized and liberated.

We have previously reported that the carbohydrate metabolism of brain is subject to endocrine influence and that oxidation of glucose accounts for only part of the oxygen utilized by brain. In the course of this study we have measured cerebral metabolic rates for other substrates. It was observed that glutamic acid is removed from blood by the brain at the rate of 0.4 mg./100 gm. of brain/minute. Simultaneously, glutamine is liberated at the rate of 0.6 mg./100 gm. of brain/minute. Krebs (1935) and Weil-Malherbes (1938) observed a similar reaction *in vitro*. They found that energy for amidation of glutamic acid is supplied by the oxidation of glucose. We investigated the effect upon this reaction of desoxycorticosterone glucoside (DCG) which interferes with the oxidation of glucose by the brain. Following the intravenous administration of DCG, a small amount of glutamic acid (0.2 mg./100 gm. of brain/minute) is still taken up but glutamine is no longer liberated. A tentative hypothesis is that the energy derived from oxidation of glucose is no longer available to permit the reaction by which the brain excretes ammonia.

OBSERVATIONS ON THE TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS. *E. L. Dubois, M.D., R. R. Commons, M.D. and P. Starr, M.D. (by invitation).* (From the Department of Medicine, University of Southern California School of Medicine, Los Angeles, Calif.)

Seventy patients with systemic lupus erythematosus (L. E.) are the basis for this report. Thirty-six were treated with various regimens including ACTH and/or cortisone. Life expectancy and the incidence of remission before and since the introduction of ACTH and cortisone have been compared. In the latter group these observations were of interest: (1) Small doses of ACTH may produce a clinical remission, yet massive doses may not be adequate in fulminating L. E. (2) If a "cushingoid" appearance is obtained there is usually a simultaneous remission of the L. E. The appearance and remission may be maintained on small doses of ACTH or cortisone. All patients showed

hypersensitivity to the immediate pressor effects of ACTH. (3) Differentiation of symptoms of L. E. from complications of therapy is important. Convulsions, psychoses and edema from either cause may have similar manifestations. (4) Female patients with active L. E. have been given massive doses of testosterone esters for as long as five weeks without the production of virilization. (5) Clinical and laboratory findings of hyperthyroidism have become apparent in two of eight patients treated with massive doses of ACTH and cortisone. (6) Nephropathy has developed in four of six patients who have been on maintenance cortisone therapy over six months. (7) The course of the disease appears to be altered by therapy with ACTH and/or cortisone. No spontaneous remissions have been seen in treated patients.

THEORETIC AND PRACTICAL CONSIDERATIONS OF THE COMBINED USE OF ACTH, CORTISONE AND CHEMOTHERAPY IN THE MANAGEMENT OF GENERALIZED PERITONITIS. *L. Boling, M.D., P. Baxter, M.D. (by invitation), J. Bertino, M.D. (by invitation), L. W. Kinsell, M.D., L. Lewis, M.D., S. Margen, M.D., A. Meyer, M.D. (by invitation), J. Newkirk, M.D. (by invitation), J. Partridge, M.D., G. Stephens, M.D. (by invitation), R. Stone, M.D. (by invitation) and R. Winkler, M.D. (by invitation).* (From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.)

Adrenal steroids appear to exert a non-specific antitoxic effect in a wide variety of toxic states. For this reason it was deemed advisable to administer ACTH and cortisone to individuals suffering from severe infections which fail to respond adequately to chemotherapy. To date they have been used in a wide variety of such infections, in many instances with salutary results. A major hazard attendant upon their use is the temporary masking of the systemic manifestations of advancing disease.

Generalized peritonitis has become a lesser hazard since the availability of potent antibiotics. Nonetheless a significant number of patients, particularly those in whom the process has progressed for several days before the chemotherapy is begun, respond poorly. The administration of ACTH and/or cortisone will result in immediate improvement in a significant number of such individuals.

On the basis of observation in one patient, it appears also that ACTH and cortisone administration to a patient with tuberculous peritonitis in whom chemotherapy alone was ineffective resulted in progressive improvement in peritoneal and pulmonary lesions.

COMBINED USE OF ACTH, CORTISONE AND CHEMOTHERAPY IN TUBERCULOUS AND NONTUBERCULOUS MENINGITIS. *J. Bishop, M.D., P. Baxter, M.D. (by invitation), J. Bertino, M.D. (by invitation), L. W. Kinsell, M.D., L. Lewis, M.D., S. Margen, M.D., A. Meyer, M.D. (by invitation), J. Newkirk, M.D. (by invitation), J. Partridge, M.D., G. Stephens, M.D. (by invitation), R. Stone, M.D. (by invitation) and R. Winkler, M.D. (by invitation).* (From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.)

In tuberculous infections it was believed that the "lytic effect" of adrenal steroids upon fibrous connective tissue might result in exposure of previously walled-off organisms to the action of streptomycin. ACTH and cortisone have been administered to several patients with tuberculous meningitis and to two patients with severe non-tuberculous meningitis who failed to respond to chemotherapy. From the data thus far obtained it appears that joint hormonal-chemotherapy, in conjunction with an adequate program of nutrition, and with constant alertness to the development of secondary infection, will result in diminution in morbidity and mortality compared to the use of chemotherapy alone.

COCCIDIOIDAL PERICARDITIS. *R. K. Larson, M.D. and R. W. Huntington, M.D. (introduced by H. E. Martin, M.D.).* (From the Kern General Hospital, Bakersfield, Calif.)

Three cases of pericarditis which we believe to be of coccidioidal origin are presented.

A forty-nine year old Negro male was admitted to the hospital because of severe substernal pain. A pericardial friction rub and the characteristic changes in the electrocardiogram established the diagnosis of pericarditis. The coccidioidal etiology was established by skin sensitivity and a positive complement fixation test. Over a nine-month period there was progressive improvement without dissemination.

A twenty-six year old Negro male was also admitted because of substernal and precordial pain. The electrocardiograms presented abnormalities compatible with pericarditis. The coccidioidal etiology was established by positive complement fixation and precipitin tests.

A sixty year old white male who was admitted because of congestive heart failure expired within a few days. At necropsy, in addition to an

extreme pulmonary emphysema and cor pulmonale, a rather remarkable chronic adhesive pericarditis was discovered. Microscopic examination of the pericardium revealed small granulomas but special stains failed to reveal any tubercle bacilli. One clear circular structure with a capsular wall was identified in a giant cell in the pericardium. This was thought to be a coccidioidal spherule and search in sections through a granulomatous lesion of the left lung revealed numerous other coccidioidal spherules. No evidence of dissemination was found in the remainder of the necropsy.

Although coccidioidomycosis has never been presented as a cause of pericarditis except as an incidental necropsy finding in disseminated cases, we believe that coccidioidal pericarditis can occur without dissemination and must be considered in the differential diagnosis of all cases of pericarditis when the patient lives or has traveled in an endemic area.

RELATIONSHIP OF POST-STREPTOCOCCAL ELECTROCARDIOGRAPHIC ABNORMALITIES TO RHEUMATIC FEVER ABD THEIR REDUCTION BY ANTIBIOTIC THERAPY. *E. O. Hahn, Major, M.C., A.U.S., G. C. Eckhardt, Capt., M.C., A.U.S. (by invitation) and D. Stowens, Capt., M.C., A.U.S. (by invitation).* (From the Streptococcal Disease Laboratory, Francis E. Warren Air Force Base, Wyoming, and the Department of Preventive Medicine, Western Reserve University School of Medicine, Cleveland, Ohio.)

A controlled study was undertaken to determine the relationship of post-streptococcal electrocardiographic abnormalities to rheumatic fever and the effect of antibiotic therapy of acute streptococcal infections on the occurrence of subsequent carditis.

Four treatment groups of patients with clinical and laboratory evidence of streptococcal infections, with simultaneous untreated control patients, were studied. Aureomycin, terramycin and two dosage schedules of penicillin were employed as therapy. Six electrocardiograms were obtained at fixed intervals over a period of three weeks after the onset of the clinical infection. Patients were questioned concerning symptoms of rheumatic fever at each of these examinations.

A definite relationship between the electrocardiographic abnormalities studied and symptoms of rheumatic fever was demonstrated. It was found that therapy over a sufficient length of time reduced not only the incidence of overt rheumatic fever but, to a lesser degree, electrocardiographic abnormalities. In the treated groups more of these abnormalities occurred without symptoms of rheumatic fever which led to the possibility that therapy masked overt rheumatic fever in many instances. It is believed that more prolonged periods of antibiotic administration will be necessary to further reduce the incidence of post-streptococcal carditis.

THE HEART IN MUSCULAR DYSTROPHY. *A. A. Sandberg, M.D. (by invitation), H. H. Hecht, M.D. and F. H. Tyler, M.D.* (From the Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah.)

Heart disease associated with muscular dystrophy has been reported but there have been no examinations of large groups. The severe physical disability of these patients makes the usual methods of cardiac evaluation impossible or meaningless and perhaps explains the infrequent occurrence of overt congestive failure which, however, has occurred in a number of instances. We have studied 400 patients with disorders of muscle; of these, sixty had cardiac abnormalities on physical, electrocardiographic and/or x-ray examination, or by cardiac catheterization studies. Our clinical findings are supported by five postmortem examinations.

Although the usual types of heart disease were observed in all groups, cardiac abnormalities occurring as a part of the muscular disease itself were observed in patients only with childhood progressive muscular dystrophy or myotonia dystrophica. This type of heart disease was not observed in patients with facioscapulohumeral dystrophy. Twenty-nine of thirty-two cases of the childhood type had persistent sinus tachycardia, definite electrocardiographic abnormalities and/or decreased cardiac excursions on fluoroscopy. Cardiac output was normal in two instances and total pulmonary and peripheral resistances were low. Extensive fibrosis and cellular changes in the myocardium were demonstrable in the absence of vascular or valvular disease.

In older subjects with myotonic dystrophy similar abnormalities were noted including electrocardiographic changes; in one instance, normal output values with low resistances were present in both circulations. In two subjects death resulted from congestive heart failure and pronounced fibrosis was found in the myocardium.

Involvement of cardiac musculature is a

common occurrence in certain types of muscular dystrophy.

PROTEINURIA IN EXPERIMENTAL RENAL DISEASE. *L. J. Rather, M.D. (introduced by E. Jawetz, M.D.).* (From the Department of Pathology, Stanford University School of Medicine, San Francisco, Calif.)

The interrelationship of proteinuria, hypertension and renal lesions has been studied in groups of rats subjected to each of the following procedures: (1) removal of one kidney, exposure and handling of the other; (2) removal of one kidney, constriction of the other with a figure-of-eight ligature; (3) removal of one kidney and one-half of the other. In rats of groups (2) and (3) there is a gradual linear increase of protein in the urine so that by the end of the fourth postoperative month the animals are excreting about fifteen and one hundred times the basal protein excretion of males and females, respectively. Male rats of group (1) show a small but definite increase in protein excretion. The development of proteinuria in these animals is independent of the development of hypertension, does not occur during the postoperative phase of restoration of lost renal tissue and in the group (3), at least, is not affected by adrenalectomy. It coincides with the development of intercapillary and extracapillary glomerular lesions with much resemblance to those seen in human glomerulonephritis, and tubular lesions characterized by the occurrence of athrocytic phenomena, focal tubular distention and atrophy.

EFFECT OF NITROGEN MUSTARD UPON CHRONIC GLOMERULAR NEPHRITIS. *R. I. Boyd, M.D. (by invitation) and R. R. Commons, M.D.* (From the University of Southern California School of Medicine, Los Angeles, Calif.)

Nitrogen mustards, spray radiation, ACTH and cortisone have been shown to alter serum complement and the course of diseases resembling glomerular nephritis in animals. Chasis, Goldring and Baldwin reported that nitrogen mustard transiently alters the proteinuria and some of the clinical manifestations of glomerular nephritis in man.

The long-term effects of nitrogen mustard upon patients with chronic glomerular nephritis in the degenerative (edematous) phase will be reported. HN_2 [methyl bis (beta chloroethyl) amine hydrochloride], intravenously, and triethylene melanine orally have been used. Dose range experience is greater for the former.

Data are presented on three patients followed longer than one year and seven followed for a shorter period. Significant rise of serum albumin concentration commencing seven to nine months after therapy has occurred in all patients followed for a year. Protein excretion in the urine has decreased transiently with the drug but a persistent decrease commenced several months later in six of seven patients with glomerular nephritis followed longer than four months. There was no alteration in one patient with amyloid disease. Persistent relief from edema has been observed in three patients followed for a year. There has been one failure to relieve edema and several recurrences that have responded to a second dose. Uremia has not been present in the group. Some patients had minimal nitrogen retention and in these there has been a slight but statistically significant reduction toward normal. One death is reported.

LIPID STUDIES IN PATIENTS WITH ADVANCED DIABETIC ATHEROSCLEROSIS. *L. T. DeWind, M.D. (by invitation), G. D. Michaels, M.D. (by invitation) and L. W. Kinsell, M.D.* (From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.)

A group of twenty-four diabetic patients with advanced atherosclerosis, many of them having lost extremities as the result of atherosclerotic involvement, were studied: (1) to evaluate the effect of various "lipotropic agents" upon diabetes as evidenced by insulin requirement and the levels of certain blood lipid components; (2) to determine whether any correlation existed between certain lipid components of the blood including total phospholipids, total cholesterol, cholesterol esters and the lipoprotein fraction denoted $S_{f\,10-20}$; (3) to determine the presence or absence of correlation between the level of the above lipid components and the atherosclerotic process.

No significant changes in the diabetic state or in blood lipids were observed in patients receiving relatively large amounts of inositol or choline. Depression of serum cholesterol esters, serum phospholipids and serum lipoprotein was observed in most patients receiving testosterone propionate in a dose of 25 mg. daily. Some changes in insulin requirement were also noted. These changes were not constant. Serum total cholesterol, lipoprotein, cholesterol ester and phospholipid values varied over a wide range. No obvious correlation between any of the lipid fractions and the atherosclerotic process was found. Linear mathematical correlation was

noted between the lipoprotein and total cholesterol; lipoprotein and cholesterol esters; cholesterol esters and serum phospholipids.

It is concluded (1) that testosterone propionate appeared to have some effect upon certain lipid constituents of the blood and (2) that there was no obvious correlation between *any* of the lipid components studied and the atherosclerotic process.

LIPOPROTEINS OF SERUM IN NORMAL AND DIABETIC CHILDREN. *C. H. Kempe, M.D., H. K. Silver, M.D., J. Carr, M.D. (by invitation), F. S. Smyth, M.D. (by invitation), J. W. Gofman, M.D. and H. B. Jones, M.D. (by invitation).* (From the Division of Pediatrics, University of California School of Medicine, San Francisco, Calif., and the Donner Laboratory, Division of Medical Physics, and Radiation Laboratory, University of California, Berkeley, Calif.)

Measurements of the concentration of $S_{f\ 11-20}$ lipoprotein molecules were made in the sera of 147 normal children ranging in age from birth to fifteen years, and in the sera of twelve women and their newborn infants. Levels of $S_{f\ 11-20}$ lipoprotein molecules were also determined in a group of forty-six juvenile diabetics.

Values obtained for the group of normal male children show them to be similar to those obtained from normal female children and approximating those for normal female adults between the ages of twenty-one and thirty years. They are significantly lower than those of normal male adults of this age group. Newborn infants had lower levels of this class of molecules than their own mothers in each instance. Levels were found to be unrelated to the daily intake of fat per unit body weight in the pediatric age group. No correlation with analytical serum cholesterol was found.

Values obtained for diabetic girls were higher than those for diabetic boys. These diabetic boys, however, have serum levels of the $S_{f\ 11-20}$ molecules higher than their normal counterparts. No correlation was found between these levels and the duration and severity of the diabetic state, dietary intake of fat or the serum cholesterol levels.

PROGNOSIS FOR SURVIVAL IN THE LEUKEMIAS OF CHILDHOOD. *H. Tivey, B.A.* (From the Department of Medicine, Division of Experimental Medicine, University of Oregon Medical School, Portland, Ore.)

The available literature has been reviewed to establish a base line for the prognosis of the survival in children with leukemia treated by methods other than the use of antifolic acid drugs, ACTH or cortisone. The distribution of the survival times from onset of symptoms to death is markedly skewed so that an "average" survival time is a misleading estimate of the prognosis for a group of such patients. The complete data on survival of a series of such cases may be readily summarized on log-probability paper and applications of this technic are made to analyze all data reviewed. From such analysis the physician can expect 50 per cent of the children with leukemia given supportive therapy (not including antifolic acid compounds—ACTH or cortisone—or the newer antibiotics on which no data are available) to live approximately four months after the onset of first definitive symptoms. About 10 per cent of his patients will survive for as long as one year. Therapy must then be planned to extend over a period of months, not merely days or weeks. At the time of review no critical evaluation of the effects of ACTH, cortisone or antifolic acid compounds on survival time of these patients could be made; neither could it be shown that the prognosis for survival of children with lymphocytic leukemia differs from that of chlldren with the less common granulocytic or monocytic leukemias.

PRODUCTION OF POSITIVE ANTIGLOBULIN SERUM TEST (COOMBS TEST) IN RABBITS BY THE INTRAPERITONEAL INJECTION OF HOMOLOGOUS BLOOD. *C. K. Liu, M.D. (by invitation) and R. S. Evans, M.D.* (From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif.)

Studies of acquired hemolytic anemia indicate that the red cell sensitizing agent is a fraction of plasma protein similar in many ways to isoantibodies, but it is not known what conditions are necessary for its production. Since it appears to be an antibody specific for human red cells, attempts to produce the disease experimentally should include attempts to sensitize the animal to an antigen in homologous red cells.

Twelve rabbits were given injections of their own blood after removal by cardiac puncture weekly or biweekly for a period of four to eight weeks. Three animals developed a transiently positive antiglobulin serum test (Coombs test) with a reagent made by injecting rabbit serum into guinea pigs (anti-rabbit guinea pig serum). Fourteen rabbits receiving injection of red cell stroma which had been incubated either in

streptococcus filtrate, rabbit kidney emulsion or rabbit liver emulsion failed to develop positive agglutination test with the same reagent. None of the injected rabbits showed evidence of hemolytic anemia.

USE OF INTRAVENOUS IRON IN IRON DEFICIENCY ANEMIAS OF CHILDHOOD. *J. C. Bramham, M.D. (by invitation), H. B. Bruyn, M.D., M. B. Olney, M.D. (by invitation) and J. P. Conrad, M.D. (by invitation).* (From the Division of Pediatrics, University of California School of Medicine, San Francisco, Calif.)

In the metabolism of iron in humans the importance of the iron reserve stored principally in the liver and spleen is often overlooked. In treating iron deficiency anemias increase in hemoglobin does not reflect the state of the iron reserves. It has been shown by Haskins, Finch and Finch that from six to twelve months are required to restore the reserves by giving oral iron. Finch has shown that intravenous iron rapidly replenishes depleted stores. Term infants exhaust the iron stored in the liver and spleen by six months of age. Children are slower than adults in replenishing the depleted storage iron, probably because of the constant large iron requirements for growth. The difficulty of maintaining iron reserves occurs especially from six to twenty-four months of age, a time when hypochromic anemias are most common. Complete therapy of hypochromic anemias should provide iron for storage as well as immediate hemoglobin formation. If the anemia is corrected but the stores are left depleted, there is little reserve to meet subsequent emergencies involving iron metabolism.

Twenty infants and children with iron deficiency anemia were studied to evaluate the effects of intravenous saccharated iron oxide. The total dosage of intravenous iron was calculated with the formula: 4.1 × weight in kg. × Hg deficit. This dosage in milligrams of iron provides 50 per cent extra for the iron reserves. Individual injections ranged from 23 to 200 mg., the initial dose being between 50 and 100 mg.

Hemoglobin responses were more rapid than those reported in adults, average daily increase being 0.33 gm. Initial hemoglobin determinations varied from 3.0 to 11.0 gm./100 ml., while final hemoglobin values were from 11.0 to 14.5 gm./100 ml. Even in the face of severe infection many showed a rapid response. Slow responses were noted only when there was severe infection and the anemia was mild. No toxic symptoms were observed.

IMMEDIATE HEMATOLOGIC EFFECTS OF INTRAVENOUS SACCHARATED IRON OXIDE IN MAN. *L. White, M.D. (by invitation), K. H. Kelly, M.D., F. Cordes, M.D. (by invitation), F. S. Brooks, M.D. (by invitation), R. L. Byron, Jr., M.D. (by invitation) and H. R. Bierman, M.D.* (From the Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, U.S.P.H.S. and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

Saccharated iron oxide administered intravenously in man is accompanied by a prompt leukopenia within five minutes. By simultaneous arterial and venous blood sampling from various points in the circulation, the leukopenia has been found to result from withdrawal of leukocytes in the lungs. The magnitude of leukocyte removal is related to the rate and amount of saccharated iron oxide infused. The granulocytes are primarily involved. The leukopenia persists for five to fifteen minutes. When blood samples were obtained downstream from the point of injection and accordingly contained large amounts of iron, the clotting power of the venous blood samples during the period of infusion was markedly enhanced. There was no comparable alteration in the platelet counts. The significance and implications of these findings will be discussed.

BLOOD FLOW IN HUMAN BONE MARROW AS DETERMINED BY THE CLEARANCE RATE OF RADIOIODINE (I^{131}) IN LEUKEMIA AND NEOPLASTIC DISEASES. *N. L. Petrakis, M.D. and S. P. Masouredis, M.D.* (From the Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, U.S.P.H.S. and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

While considerable data are available on the rates of blood flow in many organs, no clinical studies have been made on the conditions of blood flow in human bone marrow. This neglect is due, no doubt, to the technical difficulties encountered in gaining access to the blood vessels of this diffuse organ in the intact organism.

In this study the relative rates of marrow blood flow were measured by use of the Kety isotope clearance technic following the injection of radioiodide into the bone marrow in over forty patients with a variety of neoplastic

diseases and leukemias. For purposes of comparison, radioiodide clearance studies were made simultaneously in the gastrocnemius muscle. Strikingly rapid rates of radioiodide clearance (blood flow) were obtained in the marrow of patients with acute leukemia, multiple myeloma and in some patients with chronic lymphatic leukemia. In chronic myelocytic leukemia and monocytic leukemia the clearance rates were similar to those obtained in normal and non-leukemic patients suggesting that a difference exists in the state of bone marrow circulation within the leukemic group of diseases. The significance of the data obtained with respect to disease, marrow cytology, hemoglobin level, circulatory status and other factors will be discussed.

I^{131} Uptake in Treated Hyperthyroidism. *F. K. Bauer, M.D.* (From the Radioisotope Unit, Veterans Administration Center, Los Angeles, Calif.)

By means of the scintillation tube, using doses as small as one microcurie of I^{131} orally, it has become possible to repeat diagnostic tracer studies several times without hazard to the patient. Sixty hyperthyroid patients were studied. Thirty-two have been treated with therapeutic doses of I^{131}, six by surgery, four with thiourea derivatives and one with Lugol's solution. The remainder of the patients had been treated by surgery or thiourea derivatives in the past.

Previous observations by other investigators on the initial rapid rate of accumulation of I^{131} by the thyroid in hyperthyroidism were confirmed. Treated hyperthyroid patients in remission were found to have a reduced total amount of I^{131} accumulated by the thyroid but retained their rapid rate of uptake. Several patients with suspected hyperthyroidism were found to accumulate a normal maximum amount of I^{131} but to have a rapid rate of uptake. These patients are being followed clinically and by means of repeated diagnostic tracer studies to see whether the rate of uptake regresses or whether it and the total pick-up increase.

Blood Volume Expansion Produced by Dextran, Gelatin and Plasma. *G. M. Hyde, M.D. (by invitation), N. I. Berlin, M.D., L. M. Meyer, M.D. (by invitation), R. J. Parsons, M.D. and B. Whittington, M.D. (by invitation).* (From the Donner Laboratory of Medical Physics, University of California, Berkeley, Calif., and Highland Alameda County Hospital, Oakland, Calif.)

The blood volume changes following the administration of 500 cc. of dextran (6 per cent), gelatin (5 per cent), plasma and saline were studied in twenty-three patients. The blood volume was determined with P^{32} labeled red blood cells according to a modification of the method of Hevesey and Zerahn. The magnitude and duration of the blood volume expansion were determined by repeated measurements of the quantity of P^{32} per cc. of blood with correction for the amount of P^{32} lost from the cells during the period of study. These three plasma volume expanders were compared for a twenty-hour period. Dextran and gelatin gave comparable results which were greater both in magnitude and duration than those obtained with plasma. An average maximum increase in the blood volume of approximately 1,500 cc. was noted for the dextran, 1,200 for gelatin and 1,000 for plasma, with no significant change produced by saline.

Effects of Exercise on Peripheral Venous Pressure in Relation to Blood Volume, Cardiac Competence and Pulmonary Arterial Pressure in Heart Disease. *E. Brown, M.D., D. D. Daniels, M.D. (by invitation), H. R. Fluss, M.D. (by invitation), J. Hopper, Jr., M.D. (by invitation), J. D. Lange, M.D. (by invitation) and B. P. O'Connell, M.D. (by invitation).* (From the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

In ten experiments on five patients suffering from diseases involving the left ventricle primarily (group A) and in nineteen experiments on sixteen patients with either pulmonary disease or rheumatic mitral valvulitis (group B), the following procedures were carried out: After rest in recumbency for thirty minutes or more, blood volume was measured by both T-1824 and carbon monoxide methods. Venous pressure was measured continuously by saline manometer or strain gauge during five minutes of rest, five minutes in which approximately 3,500 foot-pounds of external work was performed by weight-lifting in a foot treadle and five minutes of recovery. Heart rate, respiratory rate and depth, and chest position were recorded during the same continuous fifteen-minute period. In seventeen experiments on thirteen healthy subjects, the same procedures were done, except that blood volume was not always measured.

Resting venous pressure exceeded 15 cm. saline in only three patients, all of whom had increased blood volume. In group A the rise of venous pressure during and after exercise was

significantly greater than the controls only when blood volume was expanded (20 to 60 per cent above predicted normal). Conversely, in group B no correlation was found between blood volume and the response of venous pressure to exercise, very high curves and delayed recovery being observed in several normovolemic patients. In two group B patients improved cardiac competence following quinidine conversion of auricular fibrillation was reflected by lower venous pressure curves. The presence and severity of pulmonary hypertension were reflected by the venous pressure response to exercise in five of six patients whose mean resting pulmonary arterial pressures, measured directly by venous catheter, were between 17.5 and 58.5 mm. Hg.

The results support the concept that, in cardiac patients, whereas resting venous pressure is largely dependent upon blood volume, changes in pressure resulting from exercise are dependent on the competence and work load of the right ventricle.

A Clinical and Physiologic Appraisal of Mitral Valvulotomy. *H. N. Hultgren, M.D.* (From the Departments of Medicine and Surgery, Stanford University School of Medicine, San Francisco, Calif.)

Clinical and physiologic studies have been made before and at varying intervals after surgery in selected cases of mitral stenosis in an effort to answer the following questions: (1) What proportion of patients operated upon are improved? (2) What is the degree and duration of improvement attained? (3) Does marked pulmonary hypertension adversely affect the results of surgery? (4) Does clinical improvement parallel physiologic improvement?

Studies performed in seven patients yielded the following results: (1) Five patients demonstrated distinct clinical and physiologic improvement after surgery; two patients were not benefited. (2) Improvement consisted of lowering of postoperative pulmonary artery pressures at rest and during exercise and an increase in cardiac output during exercise compared to preoperative measurements. Improvement has been sustained for over a year in two patients. (3) Two patients with severe pulmonary hypertension have been greatly improved by surgery. (4) In all cases the degree of clinical and physiologic improvement were comparable. In two cases with little physiologic benefit the degree of clinical improvement was minimal or absent.

The Possibility of Determining Total Body Water in Edematous Humans by the Administration of Deuterium Oxide Orally and the Determination of Its Concentration. *W. W. Hurst, M.D., Portland, Ore., and F. R. Schemm, M.D., Great Falls, Mont.*

Six patients with edema, four with severe cardiac disease and two children with nephritis in the so-called nephrotic stage, were given from 20 to 55 cc. of deuterium oxide by mouth. After from fourteen and a half to sixteen hours, allowed for complete distribution and equilibration, determination of the concentration of the D_2O in the blood and urine (and in either abdominal or chest fluid in four of the six cases) was carried out by methods previously described. Corrections were made for the loss of D_2O in the urine which was elaborated during the fourteen- to sixteen-hour periods. The same procedure was carried out in the six patients after the loss of their edema.

The results were not discouraging: the total loss of weight for the six patients was 42.4 kg. and the total loss of body water based on the D_2O determinations was 39.2 L.; except for one case, the differences were negligible and could be easily accounted for by a deficient caloric intake and a consequent tissue weight loss. In the four cases in which D_2O levels were obtained in pleural or ascitic fluid complete equilibration with the blood levels was noted. In only one patient the urine/blood ratio exceeded 1.025. The data of this preliminary report suggest the possibility in suitable cases of determining total body water in edematous patients by giving deuterium oxide orally and determining the concentration of the deuterium oxide in the urine.

Serum Potassium Patterns in Anuria and Oliguria. *H. R. Fluss, M.D., J. Hopper, Jr., M.D. and B. P. O'Connell, M.D. (introduced by E. Brown, M.D.).* (From the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

Critically elevated serum K concentrations have been rare in our patients with anuria and oliguria if not complicated by special circumstances known to provide a rich source of potassium to the extracellular fluids. This finding may relate to the liberal amount of glucose used in treatment.

A group of patients, regarded as showing typical serum K concentrations in the course of uncomplicated renal insufficiency accompanied

by anuria and oliguria, will be compared to a second group in whom apparently dangerous serum K concentrations were reached. Analysis of this latter group revealed (1) rather complete disorganization of the blood chemical patterns and fluid compartment relationships, and (2) frequent occurrence of unusual circumstances to which the high serum K concentrations could be related. Death occurred in several patients showing electrocardiographic patterns consistent with K intoxication, a'though serum concentrations were only about 9 mEq./L. One patient survived a moderately prolonged elevation of serum K of approximately 9 mEq./L. Electrocardiographic changes beginning to indicate K intoxication were reversed while this patient was receiving digitalis although K concentration remained essentially unchanged. Reversal of K effect with digitalis has been shown in heart-lung preparations.

When death occurs in renal insufficiency, sufficient derangements exist in the fluid volume and chemical components of the body fluid to suggest that they may well be contributory.

EFFECTS OF THE INTRA-ARTERIAL ADMINISTRATION OF EPINEPHRINE INTO THE SPLEEN. *R. L. Byron, M.D. (by invitation), K. H. Kelly, M.D., L. White, M.D. (by invitation), F. Cordes, M.D. (by invitation), A. M. Littman, M.D. (by invitation) and H. R. Bierman, M.D.* (From the Laboratory of Experimental Oncology, National Cancer Institute, National Institutes of Health, U.S.-P.H.S., Federal Security Agency, and the Division of Medicine, University of California School of Medicine, San Francisco, Calif.)

The role of the spleen in the leukocytosis following epinephrine is often attributed to the release of leukocytes from the spleen by its active contraction. A similar leukocytosis occurs, however, in some patients whose spleens have been removed.

By introducing epinephrine into the splenic artery via an intra-arterial catheter in eight patients, and sampling blood frequently and simultaneously from the pulmonary conus or hepatic vein and from a large peripheral artery, it has been possible to determine the magnitude and time relationship of leukocyte flow from the spleen and liver, into the right heart and from the lungs. Timed roentgenographic exposures with splenic arteriography have demonstrated the marked constrictive effect of epinephrine upon the splenic artery and its branches. The data suggest a major component of emptying, rather than primarily active contraction as responsible for the decrease in size of the spleen.

The findings indicate that the leukocytosis observed after intravenous or intrasplenic arterial administration of epinephrine is initially from the pulmonary leukocytic reservoir and from the spleen only in small quantities two to four minutes later.

Read by Title

COMBINED USE OF ACTH, CORTISONE AND CHEMOTHERAPY IN PULMONARY AND GENITOURINARY TUBERCULOSIS. *A. Meyer, M.D. (by invitation), G. Stephens, M.D. (by invitation), P. Baxter, M.D. (by invitation), J. Bertino, M.D. (by invitation), J. Bishop, M.D., L. Boling, M.D., L. W. Kinsell, M.D., L. Lewis, M.D., S. Margen, M.D., J. Newkirk, M.D. (by invitation), J. Partridge, M.D., R. Stone, M.D. (by invitation) and R. Winkler, M.D. (by invitation).* (From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.)

It was believed that a careful exploration of the use of ACTH and cortisone in patients with pulmonary tuberculosis in conjunction with chemotherapy should be made. To date (excepting only one patient with peritonitis, as well as pulmonary involvement) only patients with tuberculous involvement in which the prognosis was essentially hopeless have been treated. Such patients had already received an adequate trial of chemotherapy and of other conventional forms of therapy. It appears that some patients may be prepared for pneumonectomy and lobectomy who would otherwise be considered completely inoperable. Tracheobronchitis, unresponsive to chemotherapy, will improve. At present it is believed that these patients should receive relatively small dosage of ACTH or cortisone and intensive chemotherapy. It is mandatory that a high caloric, high protein intake be administered throughout the period of treatment.

Several patients with genitourinary tuberculosis, all of whom had previously received intensive chemotherapy, have received combined hormonal-chemotherapy. In all such patients there has been striking decrease in systemic toxicity and improvement in general nutrition. In some, significant and sustained improvement in the urinary findings has been observed. No instance of untoward effects in such combined therapy has been observed. The cautious use of ACTH and cortisone in conjunction with

chemotherapy may have a place in the management of patients with genitourinary tuberculosis who are refractory to other forms of therapy.

A Comparison of the Effects of Various Brands of ACTH on Normal Individuals. *H. D. Kaine, M.D. (introduced by R. R. Commons, M.D.).* (From the Department of Medicine, University of Southern California School of Medicine, Los Angeles, Calif.)

A comparison of the effects of several brands of ACTH was made on five medical students. The effects of normal saline were also studied. The preparations selected were freshly dated, mixed into solutions only when used, and refrigerated. They had previously been standardized (Sayer's rat bio-assay method) by the individual companies preparing the products. Blood eosinophil counts and hourly urinary 17-ketosteroid determinations were carried out before and four hours after the administration of the drugs. In addition, each preparation, as an unknown, was again standardized by the Sayer rat bio-assay method. The results under controlled circumstances showed an average decrease in blood eosinophils of more than 47 per cent in four of the products tested. Three of these four preparations were associated with decreases of 69, 69 and 55 per cent, respectively. The average decrease with normal saline was 26.6 per cent. The 17-ketosteroid determinations were quite variable in their results. A good response of close to 100 per cent increase was obtained with the use of every preparation but this was most consistent only with selected groups and the least consistent with normal saline. Standardization by Sayer's method showed adequate responses with each brand of ACTH. The results indicate that active batches of ACTH, quantitatively equal, as compared with the Armour standard, elicited different variable responses in a group of normal individuals. The use of hourly 17-ketosteroid determinations could be used as an added standard determining the activity of ACTH in humans but several determinations must necessarily be made due to individual variable responses.

Metabolic Studies with Methyl Androstenediol. *J. W. Partridge, M.D.* (From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.)

Three young female patients were placed on long-term studies using a chemically constant formula diet administered through a polyethylene tube. After suitable control periods, each was placed on methyl androstenediol by intramuscular or oral administration. Nitrogen, sodium, potassium and phosphorus balances and the urinary excretion of 17-ketosteroids were determined. Clinical studies were carried out in infants and chronically ill men, in patients with pituitary hypogonadism and in one female with persistent galactorrhea. The balance studies revealed a significant nitrogen, potassium and phosphorus-retaining (anabolic) effect in one patient and equivocal results in two others. The excretion of 17-ketosteroids was not increased as a result of the administration of methyl androstenediol in any case. Clinically no anabolic effects were noted in infants or chronically ill men. In large dosage (0.8–1.00 gm.) the drug was definitely androgenic and anabolic in two male patients with pituitary hypogonadism. Diminution of lactation resulted in the woman with persistent galactorrhea associated with pituitary enlargement.

The Liver in Ulcerative Colitis and Regional Ileitis. *W. E. Molle, M.D., L. Kaplan, M.D. (by invitation), J. Halstead, M.D. (by invitation) and R. Rowan, M.D. (by invitation),* Los Angeles, Calif.

Hepatic dysfunction and cirrhosis have been reported as a complication of chronic ulcerative colitis. To investigate this problem ten cases of ulcerative colitis and three patients with regional ileitis were studied by means of "liver function" tests and needle biopsies of the liver. Liver function tests include thymol turbidity, cephalin-cholesterol flocculation, bromsulfalein excretion, total proteins and A/G ratio, alkaline phosphatase, twenty-four-hour urine urobilinogen excretion and prothrombin time. These tests were performed twenty-four hours prior to the liver biopsy. In two of the three patients with regional ileitis there were no alterations in the liver function tests, and minimal increased storage of green pigmentation in the central zone of the lobule. In the third patient, who was jaundiced, changes in the liver function tests and in the liver biopsy were consistent with homologous serum hepatitis. In the patients with ulcerative colitis only two had abnormal cephalin flocculation tests, and two patients had increased urobilinogen excretion. Other tests were not abnormal. The biopsy specimens revealed slight to moderate fatty infiltration and parenchymatous degeneration and regeneration, periportal leukocytic infiltration and occasional small focal collections of mononuclear cells.

Relationship of Urinary Coproporphyrin

EXCRETION AND BODY WEIGHT. *L. A. Strait, M.D., H. R. Bierman, M.D., B. Eddy (by invitation), M. Hrenoff, M.D. (by invitation) and J. J. Eiler, Ph.D. (by invitation).* (From the University of California, San Francisco, Calif.)

The daily urinary excretion of coproporphyrins has been measured in five normal adults for eighty to ninety-eight consecutive twenty-four-hour periods and in twelve other normal subjects for shorter periods. The weights of the subjects ranged from 16 kg. to 107 kg. The mean amount of coproporphyrin excreted per day was found to vary linearly with the body weight of the adult individual, indicating that the excretion per day per unit body weight is a constant quantity. This quantity which we have called the "urinary coproporphyrin coefficient" was found to be 0.93 ± 0.04 μg. per kg. for the adults. For children it varied over a range of 0.66 to 1.50, average 1.09 μg. per kg. The successive daily amounts of coproporphyrin excreted by a normal adult individual was found to have a statistically normal distribution of values about the *mean* value. The random variations in the measured single values of daily coproporphyrin excretion were in part shown to be dependent upon variations in urine volume to the extent that they affected renal clearance of coproporphyrin and thereby the partition of excretion between urine and feces.

UTILIZATION OF INFUSED FRUCTOSE AND INVERT SUGAR IN HUMAN SUBJECTS. *J. Bertino, M.D. (by invitation), N. Dawson (by invitation), R. Greer, M.D. (by invitation), S. Margen, M.D. and L. W. Kinsell, M.D.* (From the Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Calif.)

Recently it has been reported that the infusion of invert sugar and of fructose to human subjects results in a lesser degree of hyperglycemia than when an equal amount of glucose is infused over the same period of time. The present study was undertaken to verify these observations and to evaluate some of the mechanisms involved if the differences were confirmed.

Studies have been carried out in eleven normal adult human subjects. In all instances 1,000 cc. of 10 per cent sugar solution have been infused over a period of approximately one hour. In all individuals the rise of total reducing sugar in the blood when invert sugar was infused was significantly lower than glucose. Renal excretion of reducing sugars was always greatest during infusion of glucose, and usually least with invert sugar infusion. Blood sugar levels during fructose infusion bore no constant relationship to those obtained during invert sugar infusion, but (as in the case of invert sugar) were always significantly lower than those observed with glucose infusion. Thus it seems probable that fructose is utilized (oxidized or changed to glycogen) at a much more rapid rate than glucose. The data further suggest that glucose and fructose are metabolized through quite different pathways, and that such metabolism may occur simultaneously. Studies dealing with changes in serum phosphorus and with respiratory quotient studies designed to further evaluate the mechanisms involved will be reported.

ADRENOCORTICAL FUNCTION IN CHRONIC DEPRESSED STATES AND IN CHRONIC ALCOHOLISM. *C. S. Stein, Jr., M.D. (by invitation) and R. R. Commons, M.D.* (From the Resthaven Sanitarium and the University of Southern California, Los Angeles, Calif.)

Five women with chronic depression of at least two years' duration, following rigid psychiatric and medical examinations, were investigated by Kepler tests, glucose or insulin tolerance tests, 11-oxysteroid and 17-ketosteroid excretions, epinephrin and Thorn tests, and salivary Na/K ratios as measures of adrenocortical function. Four patients revealed essentially normal resting adrenocortical function, but following a standard forty-eight hour ACTH stimulation failed to increase adrenocortical output as evidenced by 17 ketosteroid and salivary Na/K responses. One of the four, retested while in electroshock-induced clinical remission, had reverted to normal adrenocortical responsiveness. The fifth patient demonstrated a low adrenocortical resting level with normal responsiveness to ACTH. These data contrast with those of others who have reported normal adrenocortical function in psychoneurotics and normal responsiveness to ACTH in anxiety states.

The finding that acute alcoholics manifest a degree of adrenocortical insufficiency and therapeutically benefit more from adrenocortical extract than from ACTH correlates well with the observations that while four chronic alcoholic females exhibited normal resting adrenocortical function, three failed not only to increase adrenocortical output following standard ACTH stimulation but also after eight, ten and fourteen days, respectively, of intensive ACTH administration. It is significant that 17-ketosteroid excretions during the final twenty-four hours of ACTH administration in each case were less than the control level.

Case Report

Familial Mediterranean (Cooley's) Anemia Complicated by Chronic Hepatitis*

Results of Treatment with ACTH

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THE existence of mild forms of Mediterranean (Cooley's) anemia has been accepted since the familial studies of Wintrobe and his co-workers.[1] They have been reported under various names: *Cooley's trait, thalassemia minor, familial Mediterranean target-oval cell syndromes* and *hemolytic jaundice with decreased red-cell fragility* and have been reviewed comprehensively by Wolman and Dickstein.[2] The separation of the mild from the severe forms of Mediterranean anemia is not always precise since it has been shown that there is a continuous gradation in the severity of individual cases ranging from those which are asymptomatic, non-anemic or even polycythemic to those with a severe and fatal anemia.[1,3,4] Although the hereditary transmission of this familial disorder is not fully understood, it is agreed that the most severe form of the anemia occurs only in the individual whose parents both have the "trait." When only one parent has the Mediterranean "trait," the disease is almost always mild. In any one individual, however, the severity of the anemia is generally constant.[5]

Neel and Valentine[6] estimate that one in twenty-five of the Italian people of Rochester, N. Y., have the mild form of Cooley's anemia. If this is true for the millions of people of Mediterranean ancestry throughout the rest of the country, this disease should be quite a common finding. It is surprising, therefore, that there have been no reports of a case of Mediterranean anemia associated with chronic hepatitis, a disease which has been the subject of many recent reports. This may be due to the fact that since anemia may produce liver damage[7,8] and, conversely, liver disease may lead to anemia,[9,10] there is a reluctance to make two independent diagnoses when a single diagnosis seemingly accounts for all of the patient's signs and symptoms.

The treatment of severe cases of Mediterranean anemia has been disappointing. Conventional anti-anemic therapy with iron and/or liver accomplishes little and splenectomy, save for the relief of symptoms caused by the massiveness of the splenic enlargement, has been without value.[2] Repeated blood transfusion has been the only available means of controlling the anemia.

The following case is of unusual interest because of the association of mild Mediterranean anemia and chronic hepatitis with cirrhosis, the occurrence of a change in the degree of the anemia, and the results of therapy with adrenocorticotrophic hormone (ACTH).

CASE REPORT

Mrs. C., a thirty-two year old housewife, was first admitted to the First Medical Division of Bellevue Hospital in May, 1950, with jaundice, anorexia and right upper quadrant pain of ten days' duration.

Nine years prior to admission, during her first pregnancy, an enlarged spleen extending 4 cm. below the costal margin, a hemoglobin of 10 gm., a red blood cell count of 5 million, and a blood smear showing microcytosis, hypochromia, poikilocytosis, anisocytosis and a few macrocytes had been noted but no attempt was made to determine the cause of these findings. About five years prior to admission the patient had noted the gradual onset of anorexia, weakness, light-colored stools and dark urine, jaundice, right upper quadrant pain, diarrhea and the loss of 10 pounds. She remained at home without medical attention, continuing her domestic activities and, after two months, the jaundice cleared spontaneously. Al-

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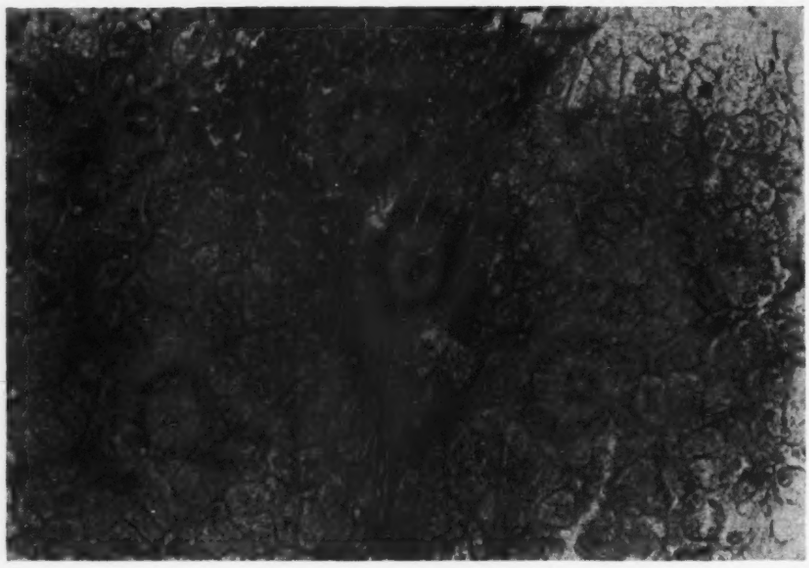

FIG. 1. Liver biopsy, 1946.

though her appetite improved she continued to suffer from weakness and fatigue. Seven months after the onset of these symptoms they became so troublesome that she was admitted to another hospital. Physical examination revealed the enlarged spleen and a firm, smooth, slightly tender liver extending 2 cm. below the costal margin. The jaundice had cleared completely. There was no fever and the blood pressure and pulse rate were normal.

Examination of the blood at that time showed a hemoglobin of 12.2 gm./100 cc., a red cell count of 4,800,000 and a white cell count of 5,700 with 47 per cent neutrophiles and 53 per cent lymphocytes. The blood smear was not described. The blood urea nitrogen was 9 mg./100 cc., and the fasting blood sugar 83 mg./100 cc. The total serum protein was 7.2 gm./100 cc. with 4.4 gm./100 cc. of albumin and 2.8 gm./100 cc. of globulin. The serum bilirubin was 1.2 mg./100 cc., the thymol turbidity 5.4 units and the prothrombin time 11.6 seconds. A bromsulfalein test showed retention of 10 per cent of the dye after thirty minutes. Examination of the urine revealed no abnormalities. The patient remained afebrile and had no complaints other than the fatigue and weakness which persisted. No specific therapy was given and she was discharged without improvement. A liver biopsy* (Fig. 1) obtained seven months after the acute episode was read as follows: "Fragments of liver include portions of two portal areas. In these zones there appears to be some increase in fibrous tissue and a mild mononuclear cell infiltration. No abnormalities of the hepatic cells are noted. The character of the specimen precludes definitive diagnosis but it may be termed 'periportal fibrosis, mild.' "*

Two years prior to admission she passed through her second pregnancy without complication and was delivered of a normal child. There was no change in her general condition during this pregnancy. Although she was noted to be "sallow," the hemoglobin was 13.6 gm. and the red cell count 4,800,000. There seemed to have been no change in the size of liver or spleen.

About five months prior to admission she had a sore throat and two weeks later noted the appearance of periorbital and ankle edema. At this time she was examined in the outpatient department where her urine was described as being dark yellow and was found to contain a heavy trace of albumin. The blood pressure was 140/90. It was believed that she had acute nephritis and hospitalization was urged but was rejected. The edema persisted for several weeks and then cleared spontaneously.

Ten days prior to admission, she developed anorexia, nausea and feverishness. Her stools became light in color, the urine dark and the skin jaundiced. She complained of a dull

* Permission to reproduce this slide was kindly granted by Dr. Mary Ann Payne, Liver Laboratory, New York Hospital.

* The pathological interpretation was written by Dr. Marvin K. Kuschner, Department of Pathology, Bellevue Hospital.

"heavy" pain in the right upper quadrant and, for the first time, noted that the ingestion of fatty foods caused abdominal distress. The weakness and fatigue which had persisted since her first attack of jaundice some four years earlier increased.

In addition to the usual childhood diseases the patient claimed that she had had typhoid fever as a child. Her subsequent health, except for an episode of mild gastroenteritis, had been excellent. She had never received any transfusions nor had she been exposed to other people with jaundice. There was no history of exposure to hepatotoxic chemicals and her diet had always been satisfactory.

Her parents had emigrated from Italy prior to her birth. Both were still living and, except for the mother's diabetes, were in good health. There were three siblings who were in excellent health and the patient had two children. There was no history of any familial diseases.

Physical examination revealed a well developed, pale, icteric woman who appeared to be chronically ill. The skin and hair appeared to be normal. No spider angiomas were noted. The features were not mongoloid. The lungs were clear. A faint blowing systolic murmur was heard at the apex but the heart was not enlarged and the sounds were regular and of good quality. The liver was enlarged; it extended 3 cm. below the costal margin and was somewhat tender to palpation. There was no ascites and the vessels of the abdominal wall were not dilated. Rectal and pelvic examinations revealed no abnormalities. The extremities were normal. There were no leg ulcers or edema. The temperature was 100°F., pulse rate and respirations were normal. The blood pressure was 130/82.

Examination of the blood revealed a hemoglobin of 8.5 gm. with a red cell count of 3,800,000. The mean corpuscular volume was 78 cubic micra, the mean corpuscular hemoglobin 22 micromilligrams, the mean corpuscular hemoglobin concentration 25 per cent. The blood smear showed considerable poikilocytosis and anisocytosis. Target cells and stippled cells were frequent and an occasional normoblast was noted. The white blood cell count was 4,750 with a normal differential count. The platelet count was 340,000. A bone marrow smear revealed hyperplasia of the erythroid elements. The Coombs test was negative.

The urine contained bile and the stools were clay-colored but these signs of biliary tract obstruction cleared after several days. Thereafter, normal amounts of urobilinogen were present in the urine. The specific gravity of casual urine specimens ranged up to 1.024 and most specimens contained small amounts of albumin. The urinary sediment revealed a few red cells and a few white cells but no casts were seen. Examinations of the stool for occult blood were persistently negative.

The blood urea nitrogen was 11 mg./100 cc. and the fasting blood sugar 86 mg./100 cc. The serum albumin was 3.9 gm./100 cc. and the serum globulin 4.2 gm./100 cc. The icterus index was 80. The alkaline phosphatase was 4 Bodansky units/100 cc. The cephalin flocculation test gave a 4 plus reaction. The blood prothrombin level was 64 per cent of normal. The serum amylase was normal.

X-rays of the chest, stomach and duodenum were normal. Esophagrams failed to reveal any evidence of varices. X-rays of the skull and long bones were normal. A flat plate of the abdomen demonstrated the enlarged liver and spleen.

The patient was treated with a high caloric diet, supplemented by vitamins and iron salts by mouth. She was given injections of liver extract, vitamin K and vitamin B complex. There was a total lack of response. The patient's symptoms, except for the jaundice which diminished, remained unchanged. The laboratory studies (Table 1) failed to show any improvement in either the hemogram or the tests of liver function. After six weeks the patient was discharged home at her request. A 500 cc. blood transfusion was given during the last week in the hospital without incident.

Following her discharge the patient resumed the bulk of her household duties in spite of continued weakness and fatigue. Mild jaundice was noted from time to time but the stools and urine remained normal in appearance. There was recurrent pain in the right upper quadrant while in the left upper quadrant there was a constant dragging sense of heaviness with occasional episodes of sharp pain and tenderness over the spleen. All of these symptoms progressed slowly until April, 1951, when the patient consented to be hospitalized for re-evaluation of her condition.

On readmission she appeared chronically ill. The skin was pale and slightly icteric. The physical examination revealed no changes other than a slight increase in the size of the liver which now extended 4 cm. below the costal

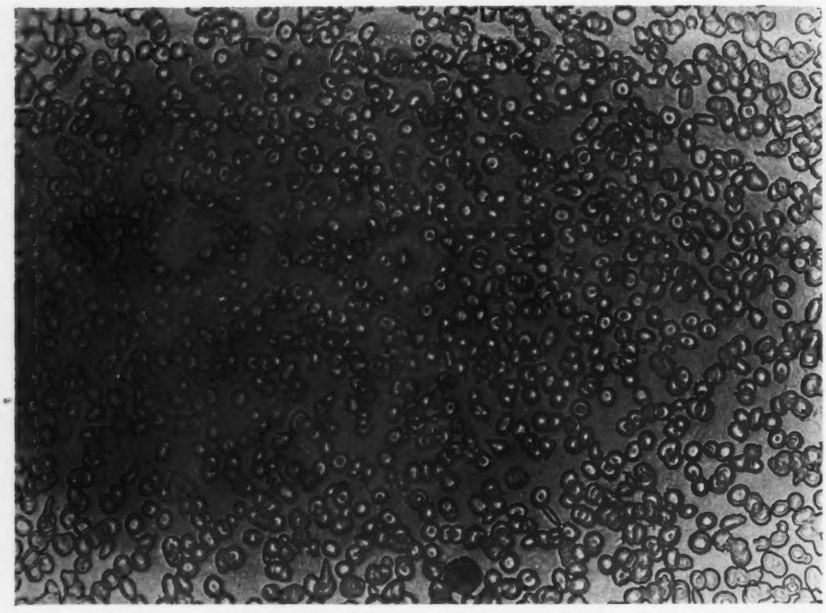

FIG. 2. Peripheral blood smear, 1951.

margin and a marked increase in the size of the spleen which now descended to the level of the iliac crest and extended almost to the midline anteriorly. The spleen was uniformly firm but there was an area of localized tenderness on the medial margin. Apart from splenomegaly, none of the signs commonly associated with portal hypertension were noted. The temperature was 99°F., the pulse and respirations were normal and the blood pressure 120/70.

The blood count revealed 8 gm. hemoglobin, 3,210,000 red blood cells, 3,600 white blood cells and 240,000 platelets. The differential count was normal while study of the red cells on smear (Fig. 2) revealed moderate anisocytosis, poikilocytosis and basophilic stippling, frequent target and oval cells, and an occasional normoblast. The mean red corpuscular volume was 78.8 cu. micra, the mean corpuscular hemoglobin 23.3 micromilligrams and the mean corpuscular hemoglobin concentration was 29.6 per cent. The bone marrow smear again showed hyperplasia of the erythroid elements. The direct and indirect Coombs tests were negative. Bleeding time, clotting time, capillary fragility and clot retraction were all normal. The red corpuscles showed increased resistance to hypotonic saline: hemolysis began at .38 per cent and was not complete at .21 per cent (determined by the method of Wintrobe[11] using washed erythrocytes) while in the control it began at .42 per cent and was complete at .38 per cent.

The daily fecal urobilinogen varied from 143 to 160 mg. (normal = 40 to 280 mg. per day given a normal total circulating hemoglobin) and the urinary urobilinogen was 6.8 mg. per day (normal < 3.5 mg. per day) by the method of Watson.[12] The hemolytic index was elevated to 40 (normal 11 to 21) indicating an increased rate of red cell breakdown.[13]

Casual urine specimens showed slight albuminuria and the sediment generally contained small numbers of red and white blood cells. The specific gravity ranged up to 1.022. A phenolsulfonphthalein test revealed normal excretion of the dye. Intravenous pyelography disclosed no abnormalities. All stool specimens contained bile while repeated tests for occult blood were negative.

X-ray studies of the esophagus failed to reveal any evidence of varices. Gallbladder x-rays were entirely normal. Duodenal drainage yielded no crystals and culture of the bile for typhoid bacilli was negative. X-rays of the skull and long bones were again normal.

The blood chemistry studies showed no significant changes from those reported on the previous admission. (Table I.) They were interpreted as indicating active liver damage with no evidence of obstruction.

It was possible to examine several members of the patient's family. (Table II.) Her mother was completely free from any evidence of Mediterranean anemia but her father's blood smear was typical of this condition and his red cells showed increased resistance to hemolysis by hypotonic

TABLE I
LABORATORY FINDINGS

	1942	1945	1946	May, 1950	June, 1950	May, 1951	May, 1951 (Pre-ACTH)	June, 1951 ACTH	July, 1951 (Post-ACTH)	Aug., 1951	Nov., 1951
Hemoglobin (gm./100 cc.)	10.0	13.6	12.2	8.0	8.2	8.0	7.7	11	11.5	10.6	10.3
RBC (millions)	5.0	4.8	4.8	3.8	3.51	3.2	3.3	3.2	4.0	4.0	4.0
Reticulocytes (per cent)	....	....	.....	4.6	1.4	6	4	6	4	....	0.2
Cephalin flocculation	....	....	.....	4+	4+	3+	4+	4+	4+	4+	4+
Icterus index	....	....	"elevated"	80	29	33	25	18	7	9	15
Serum albumin (gm./100 cc.)	....	....	4.4	3.9	2.6	3.0	2.5	2.9	2.4	....	2.2
Serum globulin (gm./100 cc.)	....	....	2.8	4.2	4.4	3.1	4.5	3.1	4.2	....	4.8
Alkaline phosphatase (Bodansky u./100 cc.)	....	....	.....	4	3	3	7	2	3	....	2.8
Bromsulfalein retention (after 30 minutes)	....	....	10%	....	.....	25%	....	35%	....	....	....
Blood prothrombin (per cent of normal)	....	....	100%	66%	84%	80%	70%	....	....	....	....

saline. One sibling was entirely normal, another showed only a rare target cell in the blood smear and it was not possible to examine the third. The patient's two children both demonstrated inheritance of this disorder. One had only a few target cells in his blood smear but had a moderately enlarged spleen for which no other explanation could be found. The second child had the typical blood smear. Neither had ever had any evidence of anemia or any history of jaundice.

The patient was treated by rest, a high caloric diet with supplementary protein, massive doses of vitamins by mouth and by injection and choline. There was no apparent response to this regimen. She continued to feel weak and tired; her appetite remained poor. The icterus persisted. There was a low-grade fever and she complained of recurrent episodes of pain localized to specific areas in both the liver and spleen. These lasted from a few hours to several days and were accompanied by tenderness in the region of the pain. There was no change in the temperature curve or in any of the laboratory findings to suggest a systemic reaction to possible inflammation or infarction.

After five weeks, a punch biopsy of the liver (Fig. 3) was done, and was interpreted as follows: "Hematoxylin and eosin, Wilder, and trichrome stained sections of liver reveal marked distortion of hepatic architecture. Small nodules of hepatic cells are separated by broad zones of fibrosis. Within the fibrous tissue there is some bile duct proliferation and small isolated groups of hepatic cells. A moderate to marked infiltration of lymphocytes and mononuclear cells is present within the areas of fibrosis. The hepatic cells have a pale granular stippled cytoplasm. Their nuclei are normal. Diagnosis: Cirrhosis of liver."*

TABLE II
FINDINGS IN FAMILY OF PATIENT

	Blood Count	Blood Smear	RBC Fragility*		Splenomegaly
			Begins	Complete	
Father	Hypochromic, microcytic polycythemia	Target and oval cells, anisocytosis, poikilocytosis	0.39	0.21	Absent
Mother	Normal	Normal	0.42	0.33	Absent
Patient	Hypochromic, microcytic anemia	Target and oval cells, anisocytosis, poikilocytosis	0.39	0.21	Present
Sister	Normal	Rare target cell	....	....	Absent
Sister	.....	.....	....	....	.....
Brother	Normal	Normal	....	....	Absent
Child	Normal	Rare target cell	....	....	Present
Child	Normal	Many target and oval cells, poikilocytosis	....	....	Absent

* Control fragility specimens began to hemolyze at 0.42 per cent with complete hemolysis at 0.33 per cent.

* The pathological interpretation was written by Dr. Marvin K. Kuschner, Department of Pathology, Bellevue Hospital.

FIG. 3. Liver biopsy, 1951.

Following the biopsy, the patient complained of pain at the site of the puncture. The temperature rose to 103°F. and she became acutely ill. She became drowsy and lethargic. Anorexia was severe and attempts to eat induced prolonged spells of nausea and vomiting. There was a slight drop in hemoglobin and red count while the icterus index rose. The other laboratory findings were unchanged. There was no evidence of intra-abdominal bleeding. This picture, it was believed, represented mild bile peritonitis and acute decompensation of the liver as a reaction to the trauma of the biopsy. The patient was kept hydrated by means of parenteral fluids and the doses of vitamins were maintained. Her condition continued to deteriorate. Finally, it was decided to administer ACTH (cortisone could not be obtained at the time). On the evening before the first dose was given, however, the patient noted spontaneous cessation of her nausea and vomiting and, for the first time since the liver biopsy, announced that her appetite had returned.

The daily dose of ACTH was divided into four equal parts which were given at six-hour intervals. The initial dose was 40 mg. per day. After three days this was increased to 60 mg. and after nine days to 80 mg. The high caloric diet with ample supplements of protein and vitamins was continued throughout. Additional doses of potassium were given.

During ACTH therapy the patient's general condition improved strikingly. Her temperature returned to normal. She ate voraciously and no longer complained of the weakness and fatigue which had lingered for years. For the first time in almost two years she became free of pain and tenderness in both liver and spleen although these organs were not observed to show any changes in size or consistency. Although there was no reticulocytosis, there was a gradual rise in hemoglobin, hematocrit and red blood cell levels. The jaundice diminished. The fecal urobilinogen ranged from 200 to 270 gm. per day and the hemolytic index was calculated as 44, indicating that there was no significant decrease in the amount of hemolysis. Studies of blood smears revealed no changes in the morphology of the erythrocytes. The tests of liver function showed no essential deviation from pretreatment levels. (Table 1.) Serial electrocardiograms revealed no evidence of hypopotassemia, the heart size did not change as measured on x-ray films and the blood pressure did not rise. Blood sodium and potassium levels remained normal and there was no evidence of disturbed sugar metabolism.

On the fifteenth day of treatment the patient was noted to be lethargic and depressed. She complained of severe headache. About one hour after her dinner she apparently fainted. At this time her blood pressure was 150/100, the heart rate 120 and respirations normal. No explanation for this episode was revealed by the clinical findings, the laboratory studies of blood sugar and electrolyte levels or the electrocardiogram.

She responded promptly to supra-orbital pressure. On the following day a similar episode occurred. Because of these and the increasing lethargy and depression, ACTH was discontinued on the sixteenth day.

Following cessation of ACTH therapy the patient's mental condition cleared rapidly. A relative sense of well being persisted and she remained free of the abdominal pain and tenderness. The improvement in hemoglobin and red count was maintained and she remained free of clinical jaundice. The abnormal liver function tests persisted. When she was discharged to her home almost three weeks following completion of her course of ACTH therapy, she presented the paradoxical picture of continuing clinical improvement with, except for the improved blood count, laboratory evidence showing essentially no change in the hemolytic process, the red cell morphology or the condition of the liver. Examination of the patient a month after her discharge revealed no changes in either her condition or the laboratory data. About four months after cessation of the ACTH therapy, however, there was a gradual return of fatigue, weakness and tenderness of the liver, while the laboratory studies revealed the return of low-grade icterus.

COMMENTS

Differential Diagnosis. The diagnosis of Mediterranean anemia in this case is based upon the classical morphological appearance of the red cells on smear, the increase in red cell osmotic resistance, the finding of the blood picture and an enlarged spleen years before any indication of liver disease, the evidence of increased red cell destruction, the characteristic failure of diet, iron and liver therapy to improve the hypochromic, microcytic anemia, and the finding of similar abnormalities in the other members of the patient's family. While certain of these features may be seen in the cases of anemia associated with liver disease, this entire syndrome has not been reported in such cases.

The diagnosis of liver disease in this case seems equally well established. The recurrent episodes of jaundice accompanied by upper abdominal pain and anorexia culminating in the continuous mild icterus with fatigue and abdominal discomfort, the hepatomegaly, and the abnormalities demonstrated by the liver function studies indicate the presence of a chronic, progressive disorder of the liver. The change from the round cell infiltration of the portal areas with no involvement of the parenchymal cells noted in the first biopsy specimen to the complete disruption of the hepatic architecture by scar tissue seen in the second biopsy specimen demonstrates the progression from an acute inflammatory process to one which is low-grade and chronic. Since there is no history or evidence of biliary tract disease, alcoholism, dietary inadequacy or exposure to hepatotoxins, the etiology is presumed to be chronic recurrent infectious hepatitis culminating in cirrhosis.

The knowledge that liver disease can cause anemia and, conversely, that anemia can cause liver disorders, suggests that in this case all of the signs and symptoms might be explained by just one of these entities. For example, it is known that the target cells in the blood smear and the increased resistance of the red cells to hypotonic saline described in the classical cases of Mediterranean anemia are not pathognomonic but may be found in patients with jaundice and liver disease.[3] Valentine and Neel[14] produced characteristic target cells from normal erythrocytes and vice versa by altering the tonicity of the solutions in which they were suspended. These cells, however, did not exhibit the increased osmotic resistance demonstrated by naturally occurring target cells. The osmotic resistance of normal red cells, on the other hand, has been increased by transfusion into patients with jaundice (merely suspending them in jaundiced serum did not have this effect).[15] It has been suggested that washing and resuspending the red cells before testing their fragility, as was done in the present case, will obviate the effect of the patient's jaundice upon their osmotic resistance.[11]

Studies of cases of cirrhosis[9] and of chronic hepatitis[10] indicate that anemia, usually of the normocytic or macrocytic type, is a common complication. Microcytic hypochromic anemia is sometimes seen but is usually attributed to chronic blood loss from hemorrhoids or esophageal varices rather than to the liver disease itself. In most instances of anemia due to liver disease, the fecal urobilinogen excretion rate in relation to the total hemoglobin is normal. When, as in this case, it is increased, it demonstrates an increased rate of destruction of the red blood cells.[16] Hemolytic anemias associated with liver disease have been described[8,17,18] but in nearly all cases the red cell fragility is either normal or increased while, almost always,

hemolysins, iso-agglutinins or Coombs antibodies can be demonstrated.[19]

It is apparent, therefore, that in the case presented the anemia could not have been attributed to the liver disease. While such an assumption would have accounted for the finding of target cells and increased red cell osmotic resistance, it could not explain the presence of hypochromic anemia in the absence of any evidence of hemorrhage or the evidence of an increased rate of red cell destruction in the face of decreased red cell fragility and negative direct and indirect Coombs tests. Furthermore, the fact that the anemia preceded the liver disease and the evidence of similar hematologic abnormalities in members of the patient's family conclusively establish the diagnosis of Mediterranean anemia. This, however, raises the question of whether the liver disease might not be a complication of the anemia. The concept that a hemolytic anemia can produce liver damage is generally accepted, although it is often difficult to be certain which came first.[8] Examination of the liver in cases of Mediterranean anemia and in both congenital and acquired hemolytic anemia has revealed fatty infiltration in the region of the central vein, granular cytoplasmic degeneration, hemosiderosis and bile-staining of the hepatic cells.[20–24] We have been unable, however, to find reported instances of cirrhosis or massive necrosis caused by hemolytic anemia in the absence of complicating factors such as septicemia[25] or cholelithiasis.[17,26] In this case the absence of any evidence of such complications and, furthermore, the fact that the episodes of jaundice, anorexia and abdominal discomfort were not produced by hemolytic crises, require rejection of this concept. It must be concluded, therefore, that the Mediterranean anemia and the liver disease were entirely separate entities.

This, however, does not preclude the possibility that each was influenced by the other. For example, the evidence that the abnormalities of the liver seen in hemolytic anemia make that organ more susceptible to injurious agents,[7] makes one wonder if, in this case, the presence of Mediterranean anemia had impaired the resistance of the liver to the virus which presumably caused the hepatitis and thereby contributed to the chronicity of the infection. This, however, is purely speculative.

The evidence that the liver disease had an adverse effect upon the anemia is more substantial. The fact that only one parent had any evidence of this familial disorder, the lack of any symptoms of anemia prior to the age of thirty and the absence of the bony disorders usually associated with the severe forms of this disease[27] indicate that this patient had a very mild form of Mediterranean anemia. Yet, as the liver disease progressed, the hemoglobin level fell from its usual 12–13 gm./100 cc. to a low of 8 gm./100 cc.

It is generally believed that the red cell and hemoglobin levels are relatively constant in the milder forms of Mediterranean anemia. Atkinson,[28] however, reported a case in which a febrile illness led to a marked increase in the severity of the anemia. Furthermore, many "trait" mothers weather pregnancy uneventfully but cases in which the anemia has increased have been described.[29–31] Hammond and Nuzum[31] attribute this to the "physiologic anemia of pregnancy" superimposed upon a "benign Mediterranean anemia." It is clear, therefore, that conditions of stress such as pregnancy or infection may intensify an anemia of the Mediterranean type. Depression of the bone marrow in cases of cirrhosis has been reported[9] and it is presumed, therefore, that in the case presented the liver disease was responsible for the accentuation of the anemia.

Treatment. The exact pathogenesis of Mediterranean anemia is not known but it is believed to be a defect in the production of red cells, probably in the synthesis of hemoglobin. Although these abnormal cells have been shown to have a decreased survival time in both moderate and severe forms of the disease, Hamilton and his co-workers have demonstrated that the occurrence of periods of partial hematopoietic arrest rather than the hemolysis is chiefly responsible for the anemia.[32] This probably explains why, except for repeated transfusions, conventional anti-anemic therapy has been without value.

Because they found adrenal hypoplasia in fatal cases of Mediterranean anemia, Whipple and Bradford[22] treated a few patients with crude adrenal cortical extract but noted no effects. Subsequently, slight elevation of the red cell count was observed in three cases following the administration of pregnancy urine.[33] Sussman[34] cites a case of Mediterranean anemia which improved following treatment with ACTH. He points out, however, that the blood trans-

fusions given during this therapy may have been responsible for the improvement.

ACTH has been used with striking success in the treatment of acquired hemolytic anemias. The response in this condition is attributed to interference with the abnormal antigen-antibody reaction causing the hemolysis. Dameshek, Rosenthal and Schwartz[19] report, however, that there is also a non-specific stimulation of red cell production or at least an acceleration of the release of erythrocytes into the peripheral circulation. A similar effect of ACTH in pernicious anemia is suggested by Sussman.[34] Davidson, Duthie, Girdwood and Sinclair[35] observed that ACTH had no effect on the rate of hemolysis in congenital hemolytic anemia but did note a moderate increase in the red cell count in one case. These observations coupled with the recent report[36] that ACTH produced a temporary improvement in patients with chronic hepatitis led us to undertake its trial in the present case.

Following the course of ACTH there was a moderate but definite increase in the hematocrit, hemoglobin and red cell levels. This should probably be attributed to non-specific stimulation of the bone marrow since there was no change in red cell morphology, the hemolytic index or, presumably, the red cell life span (the higher fecal urobilinogen level after treatment was due to the increase in circulating hemoglobin). There was a definite clinical improvement characterized by the sense of well being, increased appetite, subsidence of the fever, clearing of the icterus and disappearance of the pain and tenderness in the liver and spleen but this was belied by the absence of improvement in the liver function tests or changes in the size and consistency of the liver and spleen. The return of symptoms after four months is in accord with previous reports of the evanescence of the effect of ACTH in chronic hepatitis.

SUMMARY

A case of Mediterranean anemia in an adult aggravated by the development of chronic hepatitis and cirrhosis is reported. The effects of liver disease on the bone marrow and of anemia on liver disease are discussed. A short course of treatment with ACTH resulted in a rise in the blood count and apparent clinical improvement which were not accompanied by any improvement in the liver function tests, the hemolytic index, the blood smear or the hepatosplenomegaly. These benefits, moreover, were only temporary and probably represented a non-specific response.

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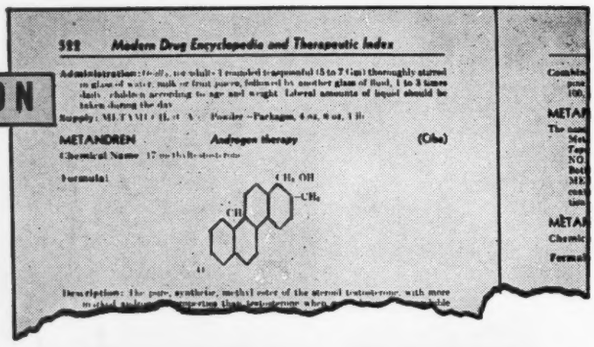

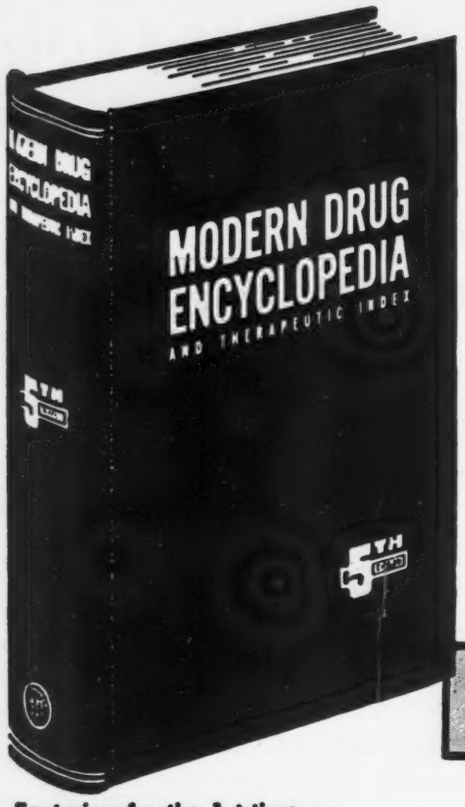

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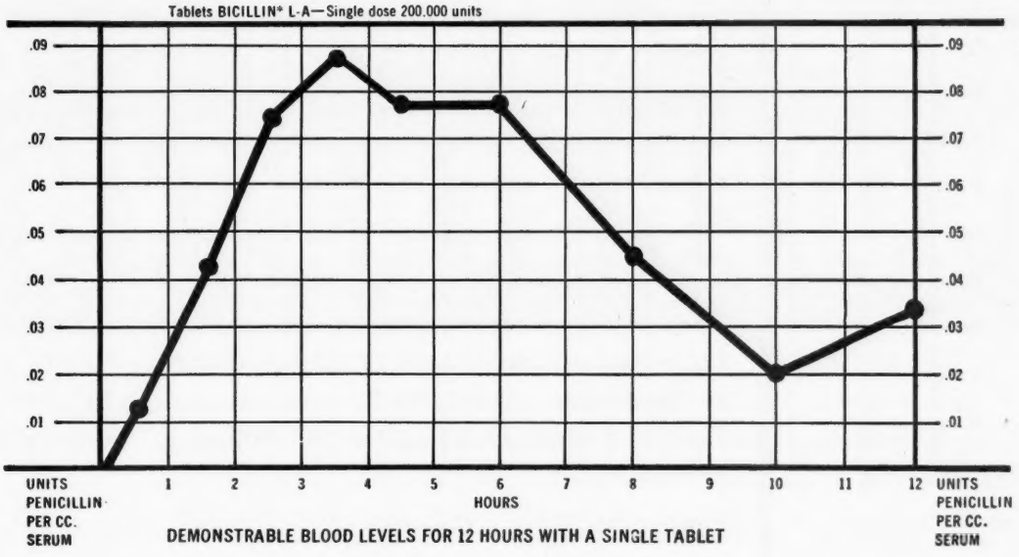

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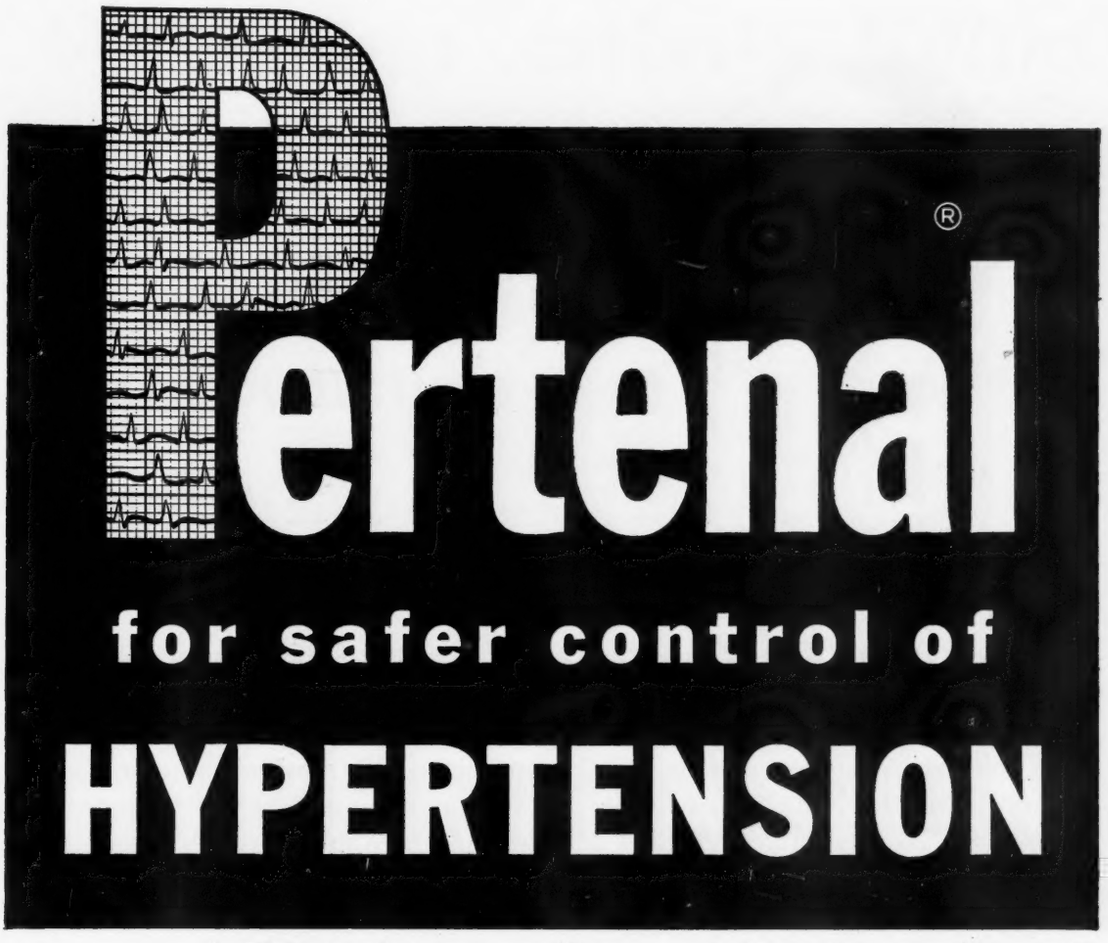

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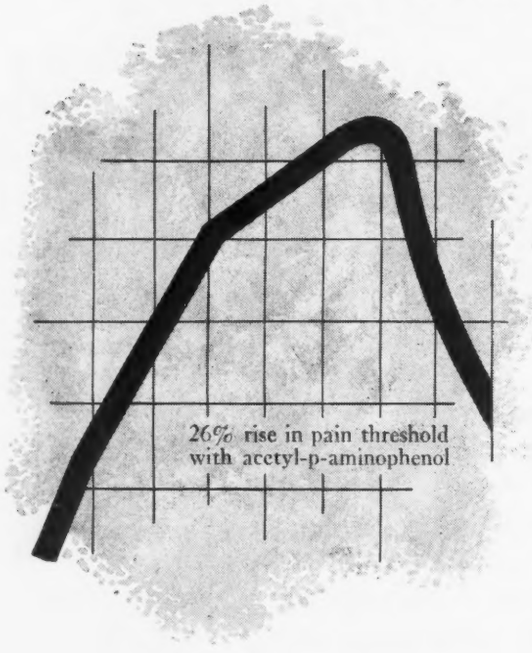

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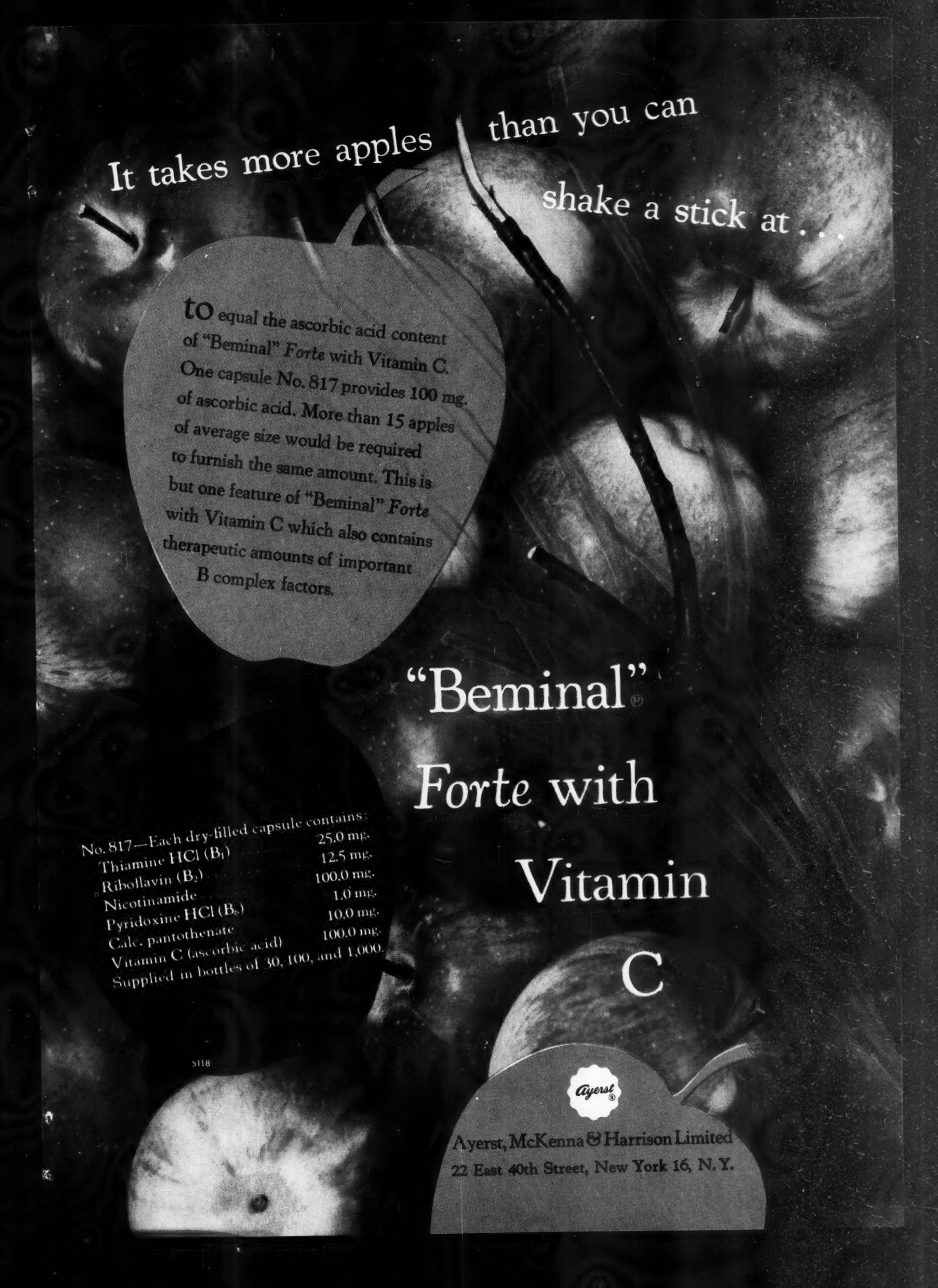
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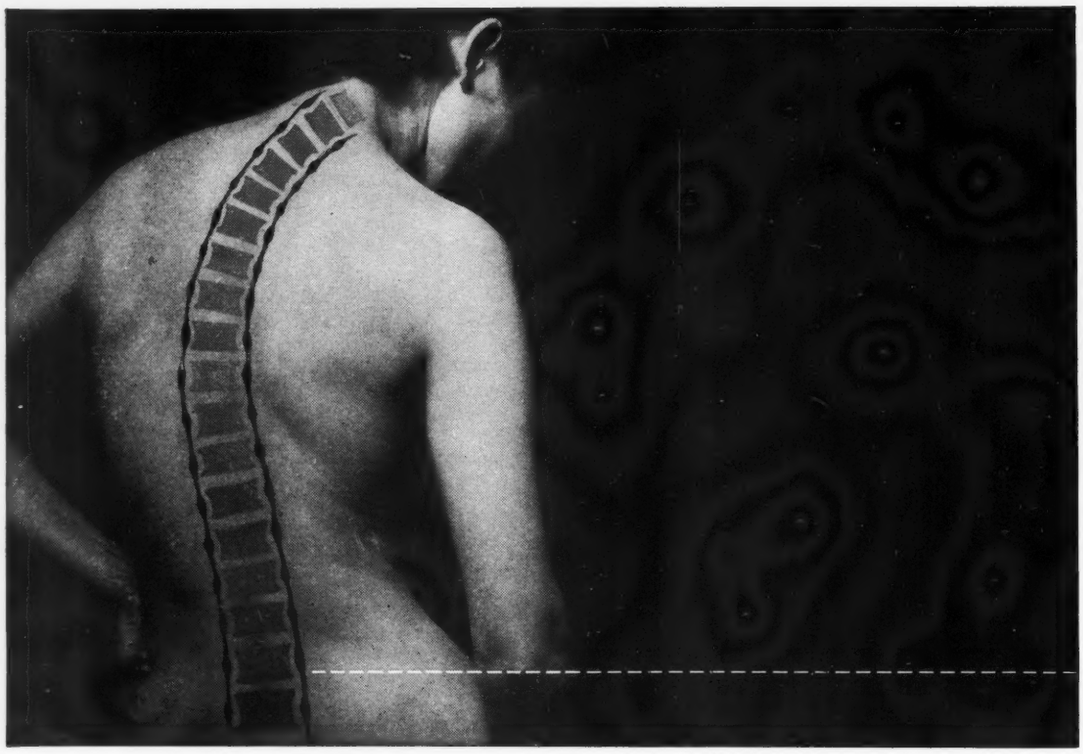

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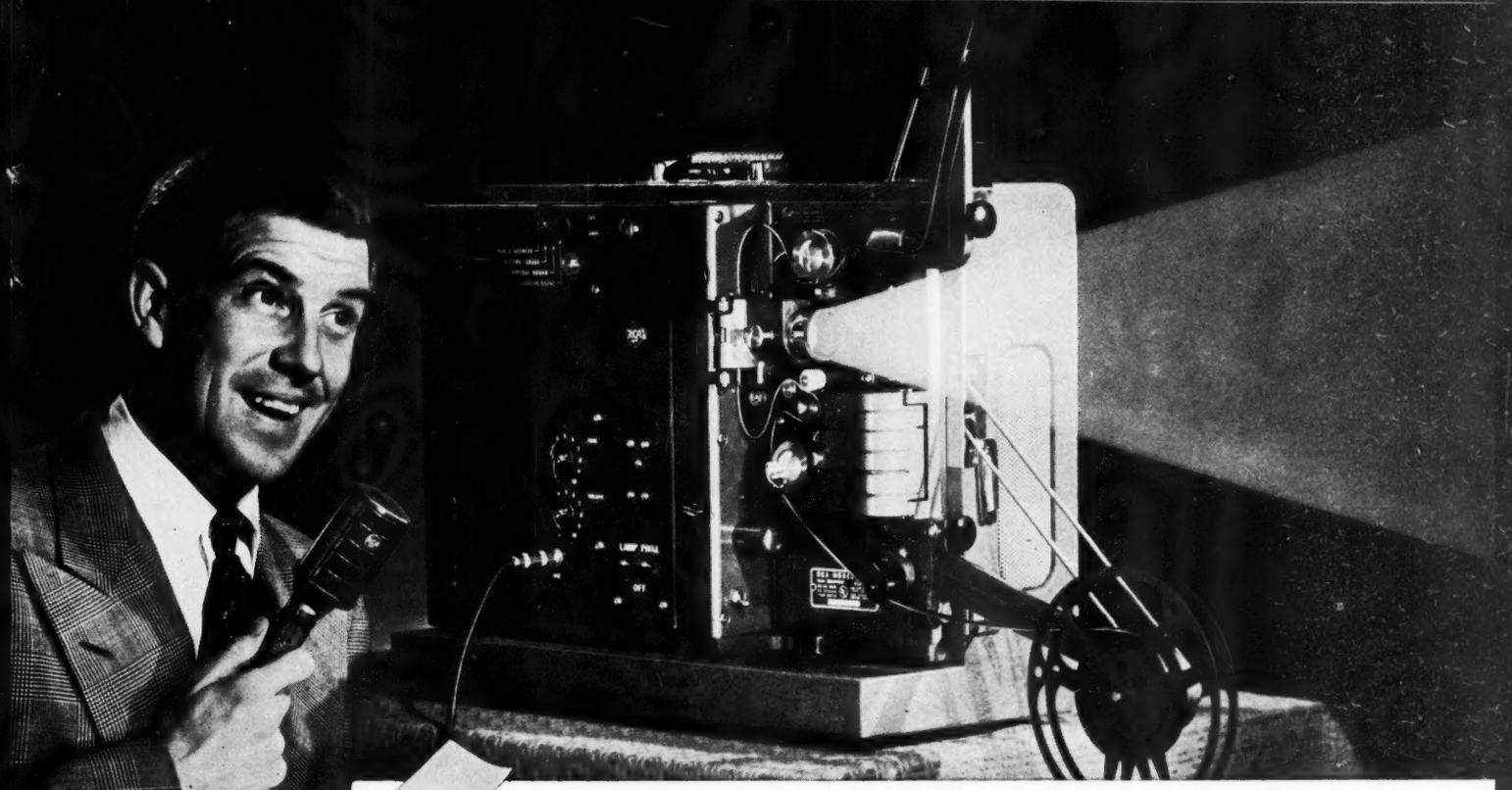

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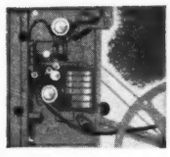

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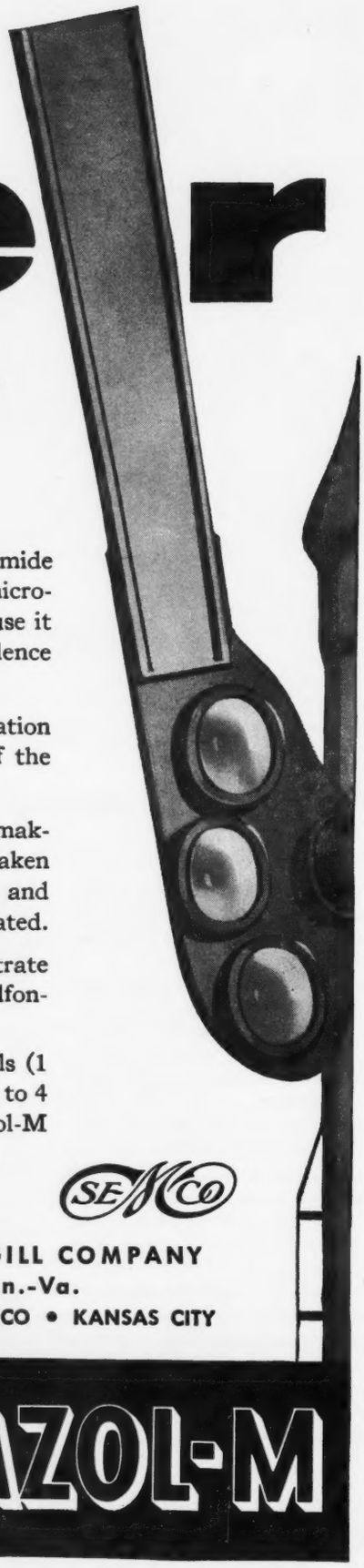

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The maintenance dose of Aldiazol-M is 2 teaspoonfuls (1 Gm. of total sulfonamides) every 4 hours; initial dose, 2 to 4 teaspoonfuls (3 to 6 Gm. of total sulfonamides). Aldiazol-M is available at all pharmacies in pint and gallon bottles.

SEMCO

THE S. E. MASSENGILL COMPANY
Bristol, Tenn.-Va.
NEW YORK • SAN FRANCISCO • KANSAS CITY

Formula

Each teaspoonful (5 cc.) of Aldiazol-M contains:

Sulfadiazine (microcrystalline)	0.25 Gm.
Sulfamerazine (microcrystalline)	0.25 Gm.
Sodium Citrate	1.0 Gm.

ALDIAZOL-M

Meat...

and Its Important Contribution of Essential Amino Acids

Although the daily allowance of protein recommended for human beings has been established for some time,[1] only very recently has a recommended daily intake of individual essential amino acids been proposed.[2] These new criteria now give a more accurate means for nutritionally evaluating the protein contribution of meat than was possible just on the basis of the gross amount of protein meat provides.

The table which follows gives the proportions of the recommended daily intake of individual essential amino acids provided by six ounces of cooked meat, the approximate average per capita daily consumption in the American diet. Note that though furnishing about 52 per cent of the daily protein allowance for a normal adult male, six ounces of meat supplies more than the recommended daily intake for a majority of the essential amino acids and a goodly proportion of the recommended intake of the remainder.

Percentages of Recommended Daily Intake of Eight Essential Amino Acids and of Protein Contributed by 6 Oz. of Cooked Meat*

Essential Amino Acids	Beef[3]	Lamb[4]	Pork[4]
L-Isoleucine	141	121	127
L-Leucine	150	120	125
L-Lysine	202	163	172
L-Methionine	42	34	40
L-Phenylalanine	70	63	70
L-Threonine	160	169	183
L-Tryptophan	90	90	100
L-Valine	136	107	113
Protein	56	49	51

*In the calculations, averages of the percentages of protein in six different cuts of each type of cooked meat were used, as were averages of the percentages of the amino acids in the protein of the respective cuts.

Every kind and cut of meat is not only an excellent source of the essential amino acids but also of the nonessential amino acids, the B group of vitamins, iron, and other essential minerals. Morever, meat is rapidly and almost completely digested, has a stimulating influence upon appetite and digestion, and gives a gratifying sense of satiety. All these nutritional and physiologic advantages of meat fully justify its prominent place in normal diets of persons of all ages and in many special diets.

REFERENCES

1. Recommended Dietary Allowances, National Research Council, Reprint and Circular Series, No. 129, Washington, D. C., 1948.
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3. Greenwood, D. A.; Kraybill, H. R., and Schweigert, B. S.: Amino Acid Composition of Fresh and Cooked Beef Cuts, J. Biol. Chem. *193*:23 (Nov.) 1951.
4. Schweigert, B. S.; Guthneck, B. T.; Kraybill, H. R., and Greenwood, D. A.: The Amino Acid Composition of Pork and Lamb Cuts, J. Biol. Chem. *180*:1077 (Oct.) 1949.

The Seal of Acceptance denotes that the nutritional statements made in this advertisement are acceptable to the Council on Foods and Nutrition of the American Medical Association.

American Meat Institute

Main Office, Chicago...Members Throughout the United States

New aureomycin minimal dosage for adults—four 250 mg. capsules daily with milk.

From among all antibiotics, Ophthalmologists often choose

AUREOMYCIN

Hydrochloride Crystalline

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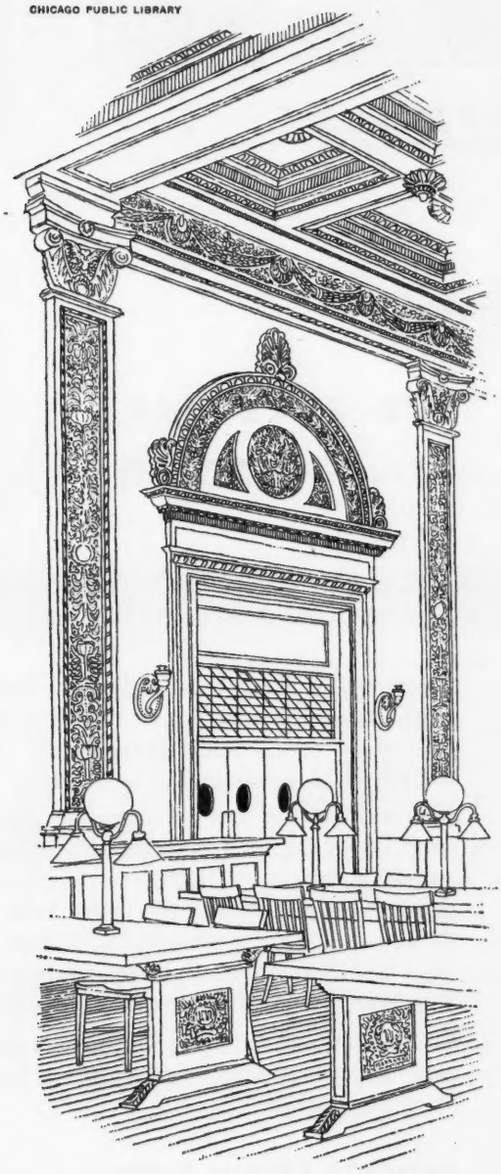


because

Aureomycin penetrates the ocular tissues and fluids, after passing the blood-aqueous barrier.

Aureomycin in 0.5% solution is well tolerated by the conjunctiva.

Aureomycin may be used locally in appropriate solution; or by mouth; or, in emergency, intravenously; or by a combined approach, depending upon the seriousness of the infection.

Aureomycin has proved of value in a number of ocular infections in which other remedies have failed.

Aureomycin has been reported to be effective against susceptible organisms in the following conditions commonly seen by ophthalmologists:

Blepharitis
Conjunctivitis
Dendritic Keratitis
Epidemic Keratoconjunctivitis
Episcleritis
Periorbital Infection
Acute Trachoma
Uveitis

Throughout the world, as in the United States, aureomycin is recognized as a broad-spectrum antibiotic of established effectiveness

Capsules: 50 mg.—Bottles of 25 and 100.
250 mg.—Bottles of 16 and 100.
Ophthalmic: Vials of 25 mg. with dropper; solution prepared by adding 5 cc. of distilled water.

LEDERLE LABORATORIES DIVISION AMERICAN Cyanamid COMPANY
30 Rockefeller Plaza, New York 20, N.Y.

Question:

WHAT MAKES Biphetacel SUPERIOR?

Answer:

THE EXCLUSIVE 1:3 L/D RATIO!

"IN CURBING APPETITE and causing weight loss, a combination of Monobasic amphetamine phosphate containing a ratio of 1:3 of levo to dextro amphetamine (as found exclusively in Biphetacel) is more effective than the same amount of amphetamine contained in the racemic form where the ratio is 1:1 l/d . . ."*

Because of its exclusive 1:3 l/d ratio, Biphetacel curbs appetite more effectively, without nausea or nervousness, in both vagotonic or "sluggish" and sympathicotonic or "high strung" patients. In addition, it preserves an "enough-to-eat" feeling by decreasing gastric motility and prolonging emptying time of stomach, and assures normal elimination by supplying evenly distributed, non-nutritive, "no clump" bulk. Small dosage means low treatment cost.

Each Biphetacel tablet contains the preferred 1:3 l/d ratio as provided by Racemic Amphetamine Phosphate Monobasic 5 mg. and Dextro Amphetamine Phosphate Monobasic 5 mg.; Metropine® (methyl atropine nitrate, Strasenburgh) 1 mg., Sodium Carboxymethylcellulose 200 mg.

Dosage: 1 tablet ½ hour before meals, three times daily, for the vagotonic type. Increase this dose, if necessary, to achieve the desired clinical results. ½ tablet ½ hour before meals, three times daily, for one week for the sympathicotonic type. If no signs of intolerance develop, increase to 1 tablet. Supplied in bottles of 100 and 1000 scored tablets.

For literature and supply for initiating treatment, write Medical Service Department, R. J. Strasenburgh Co., Rochester 14, N. Y.

*Freed, S. C. and Mizel, M.—in press

PATIENTS RETAIN THEIR ZEST FOR FOOD . . . BUT THEY

"Eat Less and Like It!"

Strasenburgh

FOUNDED IN 1886

Q.S.

is now possible

FOR LARGE DOSAGE OF ASPIRIN...

THE FIRST CLINICALLY PROVEN ENTERIC-COATED ASPIRIN

ASTERIC Brewer

ASTERIC Brewer (5 gr. enteric-coated Aspirin) ***Allows Greater Dosages***— 40, 50, 60, 70 or more grains daily as required where gastric distress and other irritating symptoms resulting from high dosages of plain aspirin tablets are contraindicated.

ASTERIC Brewer is indicated in the treatment of certain rheumatic disorders requiring maximal dosage of aspirin over long periods. *"Enteric-coated aspirin (ASTERIC) has an analgesic effect equal to that of regular aspirin and the onset of its action is only slightly delayed."* Clinically it was shown that equal blood levels were obtained.*

ASTERIC Brewer (5 gr. enteric-coated Aspirin) will be found beneficial for those patients suffering from hemorrhagic gastritis resulting from the irritating effects of plain aspirin and for cases of peptic ulcer which require acetylsalicylic acid therapy.

ASTERIC Brewer (5 gr. enteric-coated marbleized tablets) supplied in bottles of 100 and 1000.

For Samples—Just Send Your Rx Blank Marked 13AS10

*Talkov, R. H., Ropes, M. W., and Bauer, W.: *The Value of Enteric Coated Aspirin.* N.E.J. Med. 242,19 (Jan. 5) 1950.

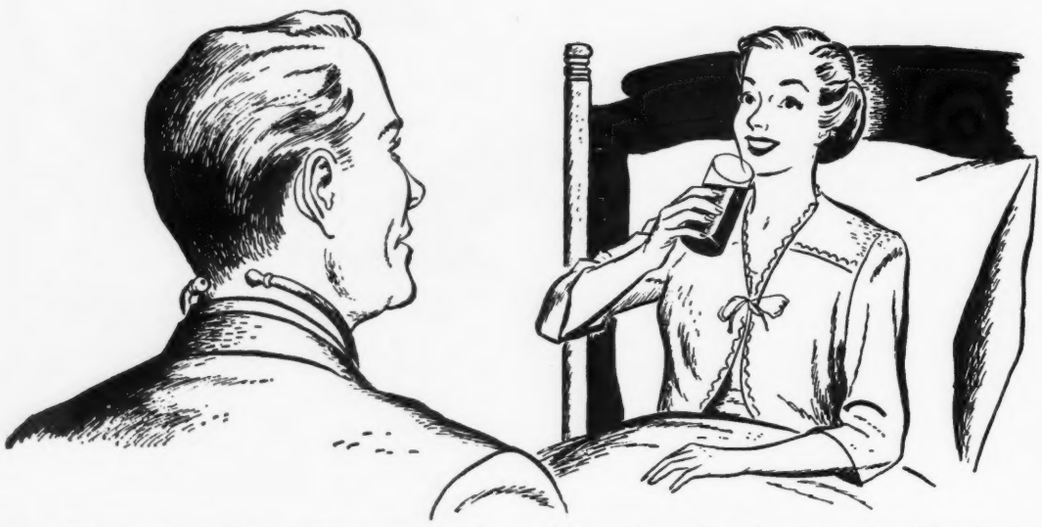
Brewer EST. 1852

BREWER & COMPANY, INC.

WORCESTER 8, MASSACHUSETTS U.S.A.

WHEN DRUG THERAPY

Increases Nutrient Requirements



The administration of many drugs can sharply increase the patient's requirements for various essential nutrients. The presence and action of certain drugs in the organism may alter normal utilization of nutrients to purposes of detoxication of these drugs.

In some instances, drugs may impair absorption of nutrients, increase their destruction within the digestive tract, interfere with their metabolism, or hasten their elimination. With prolonged administration, therefore, unless the intake of various nutrients is increased, deficiency states may be precipitated.

The dietary supplement Ovaltine in milk can significantly increase the nutrient intake of the patient when therapy makes this adjustment necessary. As shown by the table below, it provides substantial amounts of all nutrients known to be essential. Its excellent quality protein furnishes an abundance of all the indispensable amino acids.

Because of its delicious flavor, Ovaltine in milk is universally enjoyed by patients. It is easily digested, bland, and its nutrients are quickly available for utilization. The two varieties of Ovaltine, plain and chocolate flavored, both similar in high nutrient content, allow choice according to flavor preference. Children particularly like Chocolate Flavored Ovaltine.

THE WANDER COMPANY, 360 N. MICHIGAN AVE., CHICAGO 1, ILL.

Ovaltine

Three Servings of Ovaltine in Milk Recommended for Daily Use Provide the Following Amounts of Nutrients

(Each serving made of ½ oz. of Ovaltine and 8 fl. oz. of whole milk)

MINERALS				VITAMINS			
*CALCIUM	1.12 Gm.	MAGNESIUM	120 mg.	*ASCORBIC ACID	37 mg.	PYRIDOXINE	0.6 mg.
CHLORINE	900 mg.	MANGANESE	0.4 mg.	BIOTIN	0.03 mg.	*RIBOFLAVIN	2.0 mg.
COBALT	0.006 mg.	*PHOSPHORUS	940 mg.	CHOLINE	200 mg.	*THIAMINE	1.2 mg.
*COPPER	0.7 mg.	POTASSIUM	1300 mg.	FOLIC ACID	0.05 mg.	*VITAMIN A	3200 I.U.
FLUORINE	3.0 mg.	SODIUM	560 mg.	*NIACIN	6.7 mg.	VITAMIN B_{12}	0.005 mg.
*IODINE	0.7 mg.	ZINC	2.6 mg.	PANTOTHENIC ACID	3.0 mg.	*VITAMIN D	420 I.U.
*IRON	12 mg.						

*PROTEIN (biologically complete) ..... 32 Gm.
*CARBOHYDRATE ..... 65 Gm.
*FAT ..... 30 Gm.

*Nutrients for which daily dietary allowances are recommended by the National Research Council.

nothing competes with the Lure of Sweets

. . . use it in triple sulfonamide therapy

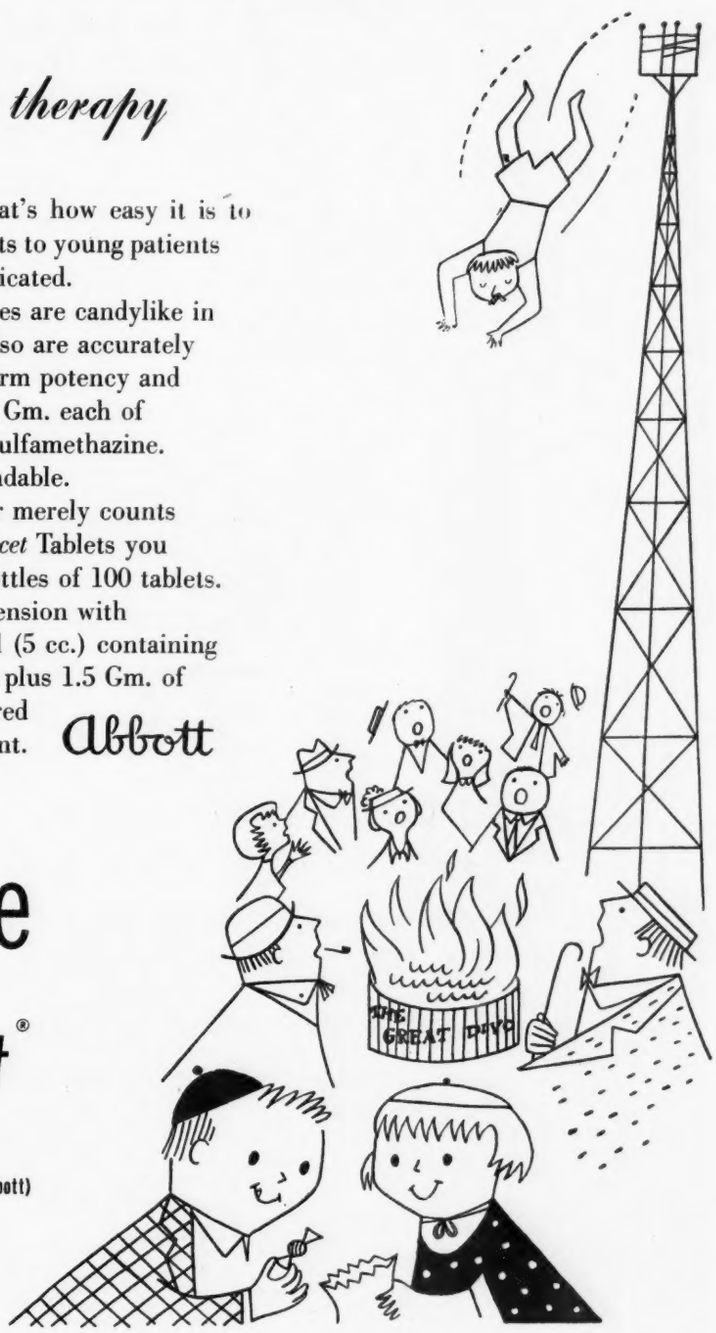
LIKE giving away candy . . . that's how easy it is to administer TRUOZINE *Dulcet* Tablets to young patients when sulfonamide therapy is indicated.

These pale-green, sugary cubes are candylike in taste and appearance, yet they also are accurately standardized medication of uniform potency and stability. Each cube contains 0.1 Gm. each of sulfadiazine, sulfamerazine and sulfamethazine.

Dosage is accurate and dependable. Administration is simple: Mother merely counts out the number of TRUOZINE *Dulcet* Tablets you prescribe. They're supplied in bottles of 100 tablets.

Also available: TRUOZINE Suspension with Sodium Citrate, each teaspoonful (5 cc.) containing 0.1 Gm. each of the three drugs, plus 1.5 Gm. of sodium citrate in a mint-flavored aqueous suspension. Bottles of 1 pint.

Abbott



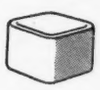
Prescribe

Truozine

TRADE MARK

Dulcet® tablets

(Moth-Dia-Mer-Sulfonamides, Abbott)

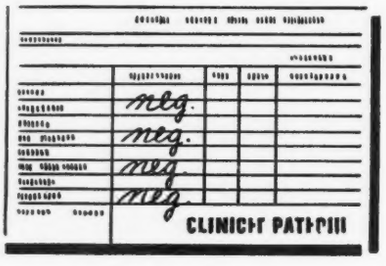


Bēplete®

VITAMINS B-COMPLEX WITH PHENOBARBITAL WYETH

For the many patients who complain of nervousness, chronic fatigue, lassitude, and anorexia

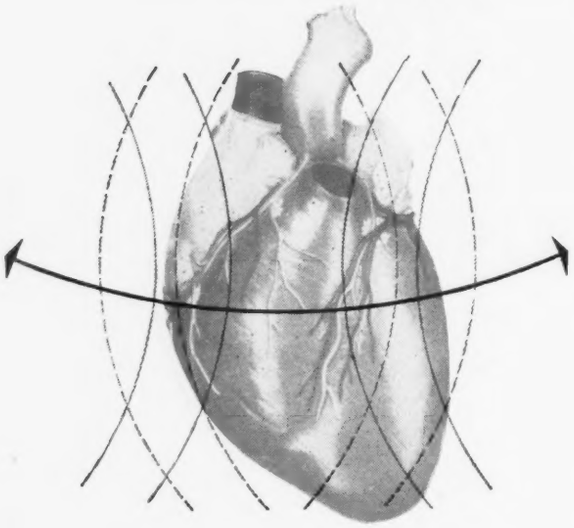
in the absence of discernible organic cause . . .



BEPLETE exerts a tranquilizing effect and provides the important benefits of high dosage Vitamin B supplementation. Available as a highly palatable Elixir, and as Tablets. Also available, **BĒPLETE with BELLADONNA** for combined antispasmodic-sedative action; Elixir and Capsule forms.

Wyeth®

In cardiac decompensation



when maintenance dosage is see-sawing...

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chief active principle of digitalis purpurea for positive, controlled maintenance

Initial compensation of the failing heart may now be accomplished in hours rather than days — but maintenance of the compensated state is often a regimen of years. Continuous adjustment of the daily cardiotonic dose, which may contribute to patient morbidity, is often obviated when a preparation of reliable, constant and unvarying potency is employed.

DIGITALINE NATIVELLE, the pioneer digitoxin, is such a preparation. It provides a uniform dissipation rate with full digitalis effect between doses. Switch your "difficult" patients to DIGITALINE NATIVELLE for smoother maintenance. Prescribe it for initial digitalization. You will be impressed with its rapidity of action and virtual freedom from local side effects.

DIGITALINE NATIVELLE is available, at all druggists, in three strengths for precise dosage — 0.1 mg. (Pink), 0.15 mg. (Blue), 0.2 mg. (White). Because of the high order of purity, most patients are adequately maintained on 0.1 mg. daily. The average dose for digitalization is 1.2 mg. in three equal doses at 4-hour intervals.

Send for brochure: "Modern Digitalis Therapy." Clinical sample available on request.

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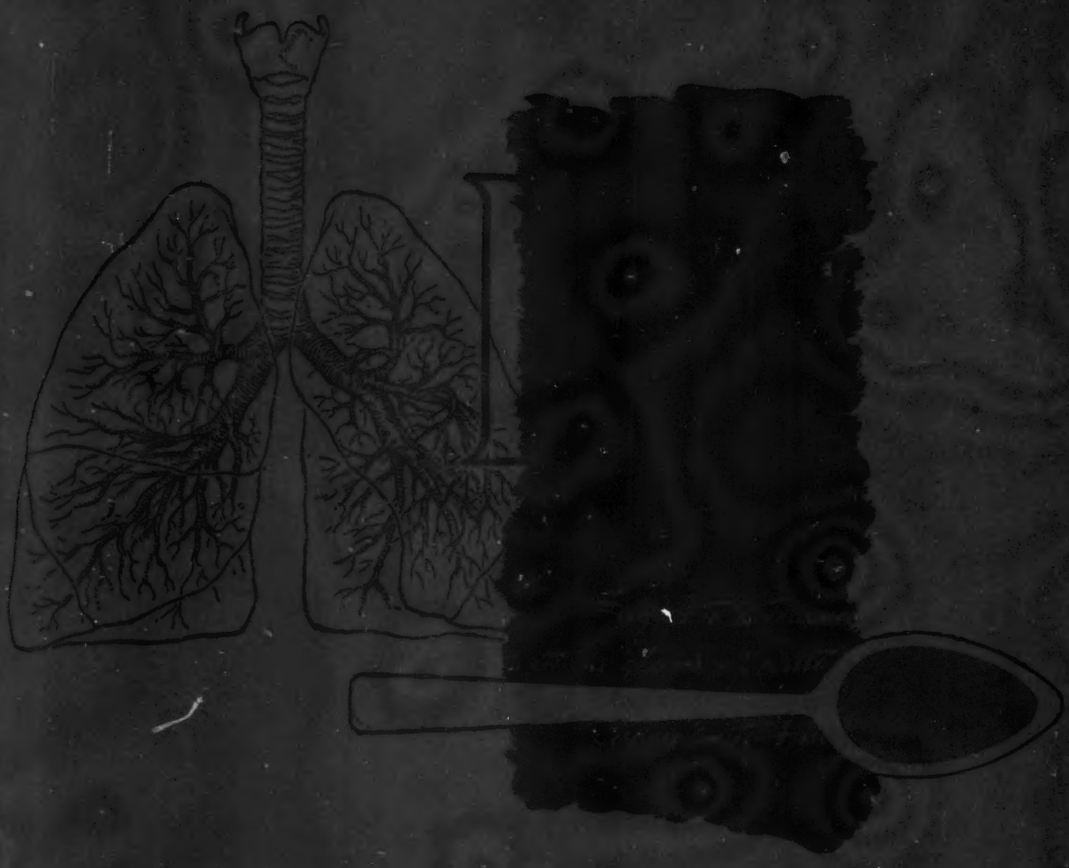
October, 1952



To Clear Congestion, Control Cough

WITHOUT CODEINE SIDE-EFFECTS

The action of a powerful histamine antagonist to relieve respiratory congestion and inflammation, alleviate bronchial irritation —this distinguishes Pyribenzamine Expectorant from ordinary cough syrups. But more than that, this unique antitussive combination provides a long-acting bronchiole-relaxant plus an effective liquefying agent to promote more productive expectoration. Pyribenzamine Expectorant thus counters basic causes of cough, without constipation or other unfavorable reactions to codeine.

● Make this non-narcotic decongestant your next prescription for cough.

Each teaspoonful (4 cc.) contains 30 mg. Pyribenzamine citrate (tripelennamine), 10 mg. ephedrine sulfate, 80 mg. ammonium chloride. In pint and gallon bottles.

Pyribenzamine® EXPECTORANT

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